AGRONOMIC POTENTIAL OF *LEUCAENA* SPECIES AND HYBRIDS IN HAWAII

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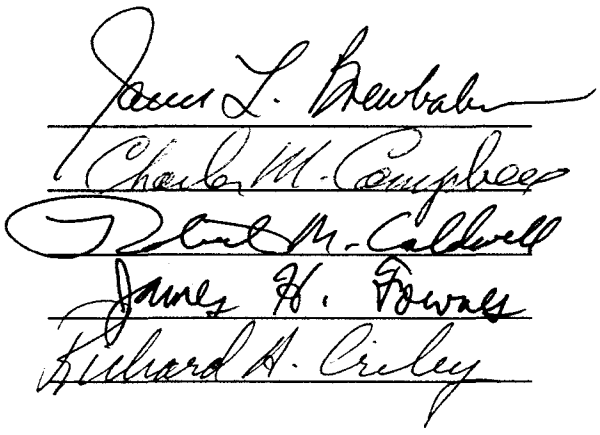
DISSERTATION COMMITTEE



EPIGRAPH

"in this pleasant soil
His far more pleasant Garden God ordain'd:
Out of the fertile ground he caus'd to grow
All Trees of noblest kind for sight, smell, taste;
And all amid them stood the Tree of Life,
High eminent, blooming Ambrosial Fruit
Of vegetable Gold; and next to Life
Our Death the Tree of Knowledge grew fast by,
Knowledge of Good bought dear by knowing ill."

John Milton
Paradise Lost
1667
Book 4, 214-222

DEDICATION

This dissertation is dedicated to my wife Yvette and son Ethan who are the center of my universe. My wife's stubborn tenacity and deep love drove me onward, while my son's trust in me provided the foundation from which to complete this chapter in my life. I also dedicate this dissertation to my father Charles P. Austin who taught me how to enjoy learning.

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ABSTRACT

A series of 10 experiments was conducted to evaluate *Leucaena* species and hybrids as forage and total biomass in Hawaii and to determine methods to vegetatively propagate this genus. Eleven *Leucaena* species comprising 84 selections (species and hybrids) were evaluated at Waimanalo, Maunawili and Mealani, Hawaii from 1991 to 1994. The best yielding forage varieties were hybrids of *L. pallida* x *L. leucocephala*. The best dry matter forage yields ranged from 18 to 22 Mg ha^{-1} yr^{-1} from 5 harvests yr^{-1} at Waimanalo and 4 to 5 Mg ha^{-1} yr^{-1} from 2 harvests yr^{-1} at Mealani. Total biomass from these trials averaged 40 Mg ha^{-1} yr^{-1} and 11 Mg ha^{-1} yr^{-1}, respectively .

Psyllid resistance was maximized in *L. pallida* varieties and hybrids with *L. leucocephala*. Other species with good psyllid resistance included *L. esculenta*, *L. collinsii*, and *L. diversifolia* K749. Cold tolerance maximized among *Leucaena pallida* x *L. leucocephala* hybrids and among *L. diversifolia* species and their hybrids with *L. leucocephala*. Wood yields maximized in the *L. leucocephala* K636 cultivar with peak mean annual increment yields ranging from 12 to 38 Mg ha^{-1} yr^{-1}. Hybrids of *L. diversifolia* x *L. leucocephala* KX3 produced 15 to 16 Mg ha^{-1} yr^{-1}. Heritability values were derived from studies of psyllid resistance and vigor. Heritability of psyllid resistance ranged from 0.37

to 0.90 for self- and open-pollinated F_4 lines of *L. pallida* x *L. leucocephala* KX2. Heritability for vigor ranged from 0.25 to 0.86. Heritability of psyllid resistance and vigor for *L. diversifolia* x *L. leucocephala* KX3 was 0.64 and 0.70, respectively, reflecting a high degree of self-fertility.

Vegetative propagation of *Leucaena* is often difficult to achieve. Sterilization procedures for micropropagation developed clean explants from field-grown plant material. Contaminated explants ranged from 0 to 40 % depending on the method used. The best results came from a 3 second dip in 90 % EtOH followed by a 7 minute dip in 20 % v/v chlorox bleach and a 7 minute dip in 0.1 % mercuric chloride. Rooting of coppiced field-grown plant material using 6 to 10 cm long one-leaf cuttings dipped in rooting hormone was successful, with 66 % of tested genotypes rooting to some degree. Rooting percentage within lines ranged from 7 to 60 %. Survivors were transplanted to the field to develop seed production orchards.

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PREFACE

The chapters that make up this dissertation will become papers of which Michael T. Austin will be the senior author. Only one paper from this dissertation has been previously published and that is Chapter 5 which was published in 1992 in Leucaena Research Reports. It is not yet known where each chapter will be submitted with the exception of Chapter 2 which is targeted for Agroforestry Systems.

CHAPTER 1

INTRODUCTION

The opportunity for improving *Leucaena* through species and hybrid selection in order to increase biomass yields of both forage and wood in Hawaii is enormous. In 1992, sales of cattle and calves in Hawaii was $29.2 million dollars, while milk sales rose to a record $32.5 million dollars (Hawaii Agric. Statstics Serice, 1993). In contrast, the average price in 1992 of imported alfalfa hay was $238 dollars Mg^{-1} (Hawaii Agric Statistics Service, 1993). It is therefore imperative to develop high-yielding, persistant *Leucaena* legume forages for Hawaii's ruminant livestock markets. Forage managed *Leucaena* can also be used for green manure in agroforestry systems for providing and recycling nutrients, or under short-rotation management schemes to provide large quantities of biomass for biofuel production. The focus of this research was to develop better *Leucaena* varieties for multiple uses for Hawaii and the developing world.

GEOGRAPHIC DISTRIBUTION OF *LEUCAENA* SPECIES

Leucaena Benth. is a member of the family Leguminoseae and subfamily Mimosoideae, and is endemic to Mexico and central America (Brewbaker *et al.*, 1990; Faria *et al.*, 1989; NRC, 1984a). *Leucaena* spp. range from Texas in the north to Ecuador in the south and colonize a wide variety of climates

and elevations (Brewbaker *et al.*, 1990). Its use as an agronomic crop dates to pre-Columbian Indian tribes of central Mexico, a fact reflected in linguistics. The state of Oaxaca, Mexico, was named after "Uaxin", a Zapotec word meaning leucaena (NRC, 1984a). *Leucaena* spp. may have been used by the Maya Indians as a nitrogen source in the form of green manure for maize production (Brewbaker, 1979). *Leucaena leucocephala* (Lam.) de Wit may have been introduced to other Pacific basin countries via established trade routes developed by the Spanish empire between the 16th and 19th centuries (NRC, 1984a). *Leucaena leucocephala* is the most widely distributed *Leucaena* species outside its native habitat (Brewbaker *et al.*, 1989). This species had been established in many tropical countries at the end of the last century to provide shade for coffee (*Coffea* spp.) and cacao (*Theobroma cacao* L.) (Brewbaker, 1987a; NFT Highlights, 1990). In addition to providing shade for cash crops, *Leucaena* is also used for wood and forage production, green manuring and soil improvement, and a variety of agroforestry uses.

SPECIES WITHIN THE GENUS *LEUCAENA*

There are sixteen species and numerous subspecies of *Leucaena* (Sorensson and Brewbaker, 1994, Table 1). Twelve species have diploid chromosome levels (2n=2x=52 or 56) while there are four recognized polyploids (2n=4x=104)

(Brewbaker 1987b; Gonzalez *et al.*, 1967; Pan and Brewbaker, 1988). Base chromosome number (x) is equal to 13 or 14 chromosomes (Brewbaker, 1987b).

Leucaena species are generally intercompatible (Sorensson and Brewbaker, 1994) with 78 and 100 % crossability reported for diploid and tetraploid levels, respectively. Incompatibility of hybridization was observed in approximately 34 % of the crosses (Sorensson and Brewbaker, 1994). Self-incompatibility has been recognized in many of the diploid species and in *L. pallida* Britton and Rose (Brewbaker *et al.*, 1990). The University of Hawaii breeding program has focused on the three polyploid *Leucaena* species, *L. leucocephala*, *L. diversifolia* ssp. *diversifolia*, and *L. pallida* because of the high intercompatibility and often superior growth of the tetraploids. Of these three polyploid species, two are self-pollinating. Self-pollinating species are *L. leucocephala* and *L. diversifolia*. They can be hybridized to create what is known as KX3. The other polyploid form *L. pallida* is self-incompatible which is postulated to be gametophytic in action (Brewbaker, 1982). *Leucaena pallida* and *L. leucocephala* can be crossed to create the hybrid KX2, while the cross between *L. pallida* and *L. diversifolia* is known as KX1. Hybridization should develop varieties that show heterosis since both *L. leucocephala* and *L. diversifolia* are inbred, and develop

Table 1.1. Somatic chromosome† number of 16 *Leucaena* spp.

Species	Chromosome #
L. collinsii Britton & Rose	56
L. diversifolia ssp. trichandra (Zucc.) Pan and Brewbaker	52
L. diversifolia (Schlecht.) Benth.	104
L. esculenta (Mocino et Sesse ex DC) Benth.	52
L. greggii S. Watson	56
L. lanceolata S. Watson	52
L. leucocephala (Lam.) de Wit	104
L. macrophylla Benth.	not available
L. multicapitula Schery	52
L. pallida Britton & Rose	104
L. pulverulenta (Schlecht.) Benth.	56
L. retusa Benth.	56
L. salvadorensis Standley	56
L. shannonii Donn. Smith	52
L. sp. "glossy" unpublished	112
L. trichodes (Jacq.) Benth.	52

†From: Sorensson, C.T., J.L. Brewbaker. 1994. Interspecific compatibility among 15 *Leucaena* species (Leguminosae: Mimosoideae) via artificial hybridizations. Amer. J. Bot. 81(2):240-247.

varieties based on several different genomes within the genus.

FORAGE PRODUCTION AND QUALITY

Forage production of *L. leucocephala* has been extensively researched, but research on other species has until recently been limited. In Hawaii, 20 to 30 Mg ha^{-1} yr^{-1} dry *L. leucocephala* forage has been reported (Brewbaker *et al.*, 1972; Guevarra *et al.*, 1978). Yields from 2 to 14 Mg ha^{-1} yr^{-1} have been reported in subtropical climates (Austin *et al.*, 1995a; Cooksley and Goward, 1988; Othman and Prine, 1984). However, since psyllids have invaded the Hawaiian Islands new emphasis was required to develop resistant varieties.

The nutritional value of *Leucaena* forage is high, with nitrogen and in-vitro digestibility averaging 2.5 to 4 g kg^{-1} and 50 to 60 g kg^{-1}, respectively (Austin *et al.*, 1995a; Tergas, 1989). Reports from Florida suggest that IVOMD is lower for *L. esculenta* compared to *L. leucocephala* (38 % vs. 55 %, respectively), an important finding since *L. esculenta* is postulated as one of the parents of the amphidiploid *L. pallida* (Pan and Brewbaker, 1988). Mineral concentration of *Leucaena* spp. are adequate for most ruminant livestock production systems with the sole limiting mineral component being sodium (Foster and Blight, 1983; NRC, 1984b; Othman *et al.*, 1985).

Antiquality factors such as mimosine and tannins are present in the forage fraction and affect feed utilization by ruminant livestock. The non-protein amino acid mimosine in forage ranges from 2 to 5 g kg^{-1} (Saunders *et al.*, 1987). Weight loss, anorexia, excessive salivation, and goiter are some of the problems associated with mimosine breakdown products, the dihydroxypyridones (Jones *et al.*, 1985; Hammond *et al.*, 1989). The discovery of *Synergistis jonesii*, has alleviated the threat of mimosine toxicity in ruminant livestock (Jones and Megarrity, 1986). Tannins are polyphenolic compounds that are thought to affect feed intake, reduce palatability (Barry and Duncan, 1984), and increase by-pass protein levels (Butler, 1989). In Australia, tannins ranged from 54 mg g^{-1} for *L. pallida* K953 to 141 mg g^{-1} for *L. diversifolia* K749. In Hawaii, Constantinides and Fownes (1994) reported tannin concentrations in *L. leucocephala* at 90 mg g^{-1}. Secondary metabolites such as tannins are thought to be one method in which psyllids are controlled.

TOTAL AND WOOD BIOMASS PRODUCTION

The apparent peak mean annual increment (MAI) for tree plantations in Hawaii is approximately 25 Mg ha^{-1} yr^{-1} (J. Fownes, personal communication), based on several reports from small-plot studies in the islands. Total DM biomass yields of six year old *Eucalyptus grandis* Hill ex Maid.

planted at a density of 3363 trees ha^{-1} had MAI's ranging from 18.4 to 26.2 Mg ha^{-1} yr^{-1} (Whitesell *et al.*, 1992). Four year old *E. saligna* at a density of 4444 trees ha^{-1} and fertilized with urea had a MAI of 23.5 Mg ha^{-1} yr^{-1} (Yost *et al.*, 1987). At higher densities of 10,000 trees ha^{-1} at Mt. View, Hawaii (elev. 389 m.a.s.l.), Dudley (1990) found *Eucalyptus* total biomass MAI to range from 28 to 36 Mg ha^{-1} yr^{-1} after 24 months.

Wood production from *L. leucocephala* K8 averaged 15 to 19 Mg ha^{-1} yr^{-1} from four year old trees at Waimanalo (Van den Beldt, 1983). Biomass yields of *L. leucocephala* ranged from 37.3 to 96.5 Mg ha^{-1} (12.4 to 32.2 Mg ha^{-1} yr^{-1}) over a three year period on four islands (Van den Beldt, 1983). Dudley (1990) reported total DM biomass yields of 2 year old *L. leucocephala* K636 at 10 Mg ha^{-1} yr^{-1} at Mt. View, Hawaii. In terms of volume, Wheeler *et al.* (1987) reported yields as high as 30 m^3 ha^{-1} yr^{-1} for four year old *L. leucocephala*. Yields of between 30 to 50 m^3 ha^{-1} yr^{-1} have been reported in tropical countries (NAS, 1980; NRC, 1984a).

Leucaena spp. may be ideal candidates for incorporation into sustainable mixed tree plantations in Hawaii because nutrients such as N can be supplied and P and K recycled through litterfall. Studies have shown that 210 kg ha^{-1} yr^{-1} N maximized *Eucalyptus* growth (Yost *et al.*, 1987). Whitesell *et al.* (1988) reported that N fertilizer was not necessary in mixed *Eucalyptus/Paraserianthes falcataria* (L.)

Nielson stands at Hamakua, Hawaii after year one. Litterfall biomass in mixed *Eucalyptus/Falcataria* plots averaged 13 Mg ha^{-1} yr^{-1} supplying approximately 110-140 kg N and 6-7 kg P ha^{-1} yr^{-1} (Binkley *et al.*, 1992).

FACTORS LIMITING PRODUCTION

Natural limiting factors to biomass production of leucaenas include psyllid susceptibility (Austin *et al.*, 1995b; Glover, 1987), low acid soil tolerance (Hutton, 1985; Oakes and Foy, 1984), poor growth and mortality in low temperatures (Austin *et al.*, 1995a; Glumac, 1986; Long, 1989), and slow establishment in response to weed competition (Cameron, 1988; Wildin, 1985).

The University of Hawaii *Leucaena* breeding program has concentrated on utilizing existing variation in the different species in order to maximize productivity of wood and forage. Most notably, variation found at the tetraploid chromosome level has been exploited since these species are highly intercompatible (Sorensson and Brewbaker, 1994). Psyllid resistance and cold tolerance have been the main objectives in Hawaii.

The most important breeding objective in the last 10 years has been for psyllid (*Heteropsylla cubana* Crawford) resistance. The leucaena psyllid is an insect pest native to Mexico and Central America that probably co-evolved with *L. leucocephala*. Psyllids arrived in Hawaii in 1984

(Nakahara and Lai, 1984) and caused extensive damage to susceptible *Leucaena* throughout the island chain. Psyllids are members of the order Homoptera and family Psyllidae (Beardsley, 1986), which infest the young, actively growing shoots and leaves of the plant, feeding and ovipositing in these areas (Beardsley, 1986). Variability for resistance in the genus is extensive. High resistance has been identified in *L. pallida*, *L. esculenta*, and *L. collinsii* (Austin *et al.*, 1995b; Glover, 1987). The polyploid *L. pallida* is best suited to developing resistant hybrids with *L. leucocephala*. The F_1 hybrid KX2 between *L. pallida* x *L. leucocephala* has shown decreased damage by psyllids in Florida and Hawaii (Austin *et al.*, 1995b; Wheeler and Brewbaker, 1989). Resistance is much more variable in the F_2 and later generations of this hybrid. New hybrids recently developed have shown great promise for increased biomass yields. The new KX2 varieties such as K748 x K636 may produce even greater yileds than either parent or the older KX2 cross between K376 x K8.

While investigating F_2 KX3 progeny (*L. diversifolia* x *L. leucocephala*), Pan (1989) reported that a normal distribution for psyllid resistance occurred, suggesting control by several genes. Morphological characteristics such as leaflet size or mucilage production may be partly responsible for resistance (Sorensson, 1989; Sorensson and

Brewbaker, 1986). However, mechanisms for psyllid resistance are still poorly understood.

The other breeding objective is to develop cold-tolerant *Leucaena* species and hybrids for higher elevations in the tropics, and for lowland subtropical environments. Breeding for cold tolerance is warranted in order to extend the range of economically feasible *Leucaena* biomass yields from relatively narrow tropical lowland ecozones to higher elevations. There are basically two types of cold tolerance that need to be addressed. The first is to develop lines that grow well in cooler environments with no frost hazard. Kamuela, Hawaii, is a good example of an environment with no frost hazard at 900 m with approximately 6 to 7 ^{0}C cooler maximum temperatures than Waimanalo. The other environment is where the growing season for *Leucaena* is restricted by frosts. The subtropical environment of central Florida is a good example where the *Leucaena* growing season is approximately 6 months, and where frosts break the psyllid pest cycle (Austin *et al.*, 1995a; Austin *et al.*, 1995b). Several *Leucaena* species exhibit tolerance to cooler temperatures *L. diversifolia*, *L. retusa*, *L. greggii*, and *L. pallida* show better growth at higher elevations than *L. leucocephala* (Brewbaker *et al.*, 1988). Interspecific hybridization of *L. diversifolia* x *L. leucocephala* (KX3) produced trees with superior cold tolerance at elevations of 900 m at Mealani, Hawaii compared to the complete failure of

the *L. leucocephala* parents (Brewbaker *et al.*, 1988). The other parent in the cross *L. diversifolia* K156 performed almost as well as the hybrid. Field and greenhouse studies in Texas and Arizona report better frost tolerance of *L. retusa* as compared to *L. leucocephala* (Glumac, 1986; Long, 1989). Winter survival of eight *Leucaena* lines grown in Florida between 1989 to 1991 ranged from 48 to 62 % after the first winter in which temperatures dropped below freezing for three consecutive days (Austin *et al.*, 1995a).

The other factors limiting biomass production of *Leucaena* include low acid soil tolerance and slow seedling establishment and are important considerations for developing improved *Leucaena* lines for multiple environments. *Leucaena* species and hybrids differ with respect to acid soil tolerance (Hutton, 1985; Oakes and Foy, 1984). Acid soil tolerance involves the improved capability of plants to take up calcium in soils with high aluminum concentrations (Hue *et al.*, 1986; Hutton, 1989). *Leucaena leucocephala* is considered to be intolerant to soil pH below 5.0 (Hutton, 1989), while *L. diversifolia*, *L. macrophylla* and *L. shannonii* are reported to be tolerant (i.e. grow better) to acid soils (Hutton, 1981). In Brazil, KX3 had better acid soil tolerance than the *L. leucocephala* parents (Hutton, 1989).

Improved seedling establishment is important for controlling weed infestations in *Leucaena*. *Leucaena*

leucocephala is a slow starter and is often the reason why leucaena plantings in Australia fail (Lesleighter and Shelton, 1986). Sorensson *et al.* (1994) identified several *Leucaena* species and hybrids with better vigor (vigor as a measure of above ground growth and biomass after 84 days) than *L. leucocephala*. *Leucaena pallida* species and hybrids had twice the early growth of *L. leucocephala* cv Cunningham or common leucocephala (Sorensson *et al.*, 1994).

CONCLUSION

Leucaena spp. are versatile, multi-purpose trees and shrubs that are used for a variety of agricultural practices. *Leucaena* spp. are highly intercompatible, allowing breeders to capitalize on mating combinations that improve yields and exhibit good psyllid resistance and cold tolerance. The better environmental adaptability of the F_1 progenies may be the result of the diverse genomic character of these hybrids. Tetraploid F_1 hybrids may have 4 diverse genomes since it is believed that both parents are amphidiploid (Pan and Brewbaker, 1988). Hybrids of *L. pallida* x *L. leucocephala* KX2 and *L. diversifolia* x *L. leucocephala* KX3, also have other favorable attributes like improved acid soil tolerance and seedling vigor.

CHAPTER 2

FORAGE DRY MATTER YIELDS OF THIRTY-ONE *LEUCAENA* VARIETIES AT WAIMANALO, HAWAII

ABSTRACT

Since psyllids (*Heteropsylla cubana* Crawford) invaded the Hawaiian Islands in 1984, forage yields of *L. leucocephala* have been reduced as the result of damage. The forage productivity and psyllid resistance of 31 *Leucaena* species and interspecific hybrids were assessed from 5 harvests in Hawaii during 1991 and 1992. We tested whether *L. pallida* species and F_1 hybrids with *L. leucocephala* seedling vigor was maintained over time. The trial consisted of an augmented randomized complete block with 22 *Leucaena* selections in all 4 replicates, 2 selections in 3 replicates, and 7 selections in 1 or 2 replicates.

Forage (leaves and small stems < 6 mm diam.) dry matter (DM) biomass yields over a 13 month period ranged from 1.4 to 34 Mg ha^{-1} while total DM ranged from 1.9 to 63.7 Mg ha^{-1}. Percent edible fractions ranged from 49 to 78 % (forage DM/total DM). The 10 varieties in this trial based on *L. pallida* Britton & Rose, and its hybrids with *L. leucocephala* (Lam.) de Wit consistently produced both the highest average forage and total DM yields averaging 22 and 40 Mg ha^{-1},

respectively. This represented a three-fold increase in forage production when compared to *L. leucocephala* K636.

The excellent yield performance of the *L. pallida* lines was attributed to high psyllid resistance and seedling vigor. All *L. pallida* selections with the exception of K953 exhibited good psyllid resistance. *Leucaena diversifolia* Benth. K749, *L. pallida* K376, and *L. esculenta* (Moc. & Sesse) Benth. K950 had the highest psyllid resistance ($P<0.05$). Forage and total DM were positively correlated to seedling vigor for the first harvest and combined harvests.

INTRODUCTION

Tree and shrub forage legumes are increasingly being emphasized for ruminant production in tropical and subtropical regions (Shelton *et al.*, 1991). An estimated 35,000 ha of leucaena has been planted for cattle forage in Central Queensland, Australia (Middleton *et al.*, *In press*). In many instances, legume trees are the only source of protein in tropical grasslands because of the difficulties in establishing and maintaining herbaceous legumes, notably during drought. *Leucaena leucocephala* is highly regarded as a ruminant livestock forage and is probably the most commonly grown forage tree legume species in the world (Brewbaker *et al.*, 1989).

In Hawaii up to 30 Mg ha^{-1} yr^{-1} forage DM was reported for the best yielding *L. leucocephala* selections prior to

the invasion of the psyllid in 1984 (Brewbaker *et al.*, 1972). Total DM biomass yields of 20.8 Mg ha^{-1} yr^{-1} (12.3 Mg ha^{-1} yr^{-1} forage DM) of *L. leucocephala* K8 at 45,000 plants ha^{-1} under psyllid-free conditions have been reported for the island of Hawaii (Guevarra *et al.*, 1978). Total DM yields were highest during periods of high solar radiation and high night temperatures (Guevarra *et al.*, 1978). Percent forage of K8 on Hawaii was 58 % when harvested at 1.5 m above ground level (Guevarra *et al.*, 1978). In Florida, percent edible of eight *Leucaena* spp. harvested five times/year ranged from 62 to 94 % (Austin *et al.*, 1995a).

Forage-managed *L. leucocephala* provides optimal conditions for psyllid infestations. Psyllids infest the young, actively growing tips of the plant, feeding and ovipositing in these areas (Beardsley, 1986). Severe psyllid infestations of susceptible *Leucaena* spp. have been reported in the Pacific Basin, Southeast Asia and Australia (Mitchell and Waterhouse, 1986). Psyllids have recently invaded the African continent (Van den Beldt and Napompeth, 1992) essentially circumnavigating the earth.

Genetic variability for psyllid resistance is extensive (Brewbaker *et al.*, 1989). Psyllid resistance has been identified in *L. pallida*, *L. esculenta* and *L. collinsii* Britton & Rose (Austin *et al.*, 1995b; Bray and Woodroffe, 1988; Sorensson and Brewbaker, 1986). Single cross F_1 hybrids produced from the mating of *L. pallida* x *L.*

leucocephala (KX2) exhibited increased psyllid resistance compared to *L. leucocephala* (Sorensson and Brewbaker, 1986).

The purpose of this study was to survey a broad range of *Leucaena* spp. and hybrids for forage productivity in a tropical, lowland environment to determine if the new hybrids currently being developed at the University of Hawaii are superior in psyllid resistance and yield.

MATERIALS AND METHODS

The Waimanalo Research Station is located at 21° 20′ N, 158° 20′ W with a mean elevation of 20 m above sea level. Precipitation average 1380 mm yr^{-1}, and mean annual temperature is 24.6^0 C. Precipitation, temperature and solar radiation were measured during this experiment (Appendix A). Temperature was converted into growing degree days (GDD) based on the sum of the maximum temperature minus the minimum temperature divided by 2 minus 10^0 C over the duration of each harvest interval. Both GDD and mean monthly solar radiation were used to test correlations with forage DM yield (Appendix B).

The 31 varieties in this trial were selected to encompass several previously untested species and newly developed F_1 hybrids, against what are considered commonly used varieties. Seeds from 31 varieties (Table 2.1) were scarified in concentrated H_2SO_4 for seven minutes, rinsed, and soaked in tap water overnight. Seeds were inoculated

Table 2.1. Species grouping, parentage, and label of 31 *Leucaena* selections in Groups I and II.

Species Parentage†	Label
GROUP I	
L. pallida K376	PALL 376
L. pallida K804	PALL 804
L. pallida K819	PALL 819
L. pallida K953	PALL 953
L. pallida x *L. pallida* (K806 x K748)	PALL HYB
L. pallida x *L. leucocephala* (K806 x K636‡)	PxL 1
L. pallida x *L. leucocephala* (K748 x K636)	PxL 2
(*L. diversifolia* x *L. leucocephala*) (K156 x K8) x *L. pallida* x K806	(DxL)xP
(*L. pallida x L. diversifolia*) (K748 x 156) x *L. leucocephala* x 636	(DxP)xL
L. pallida x *L. diversifolia* (K806 x K156)	PxD
GROUP II	
L. collinsii K450	COLL 450
L. collinsii K905	COLL 905
L. collinsii K911	COLL 911
L. diversifolia (2n) K749	DIV2 749
L. diversifolia (2n) K919	DIV2 919
L. diversifolia (4n) K156	DIV4 156
L. diversifolia (4n) K146	DIV4 146
L. diversifolia x *L. leucocephala* (K156 x K636)	DxL
L. esculenta K948	ESCU 948
L. lanceolata K393	LANC 393
L. leucocephala K500 (var. Cunningham)	LEUC 500
L. leucocephala K636	LEUC 636
L. leucocephala K997	LEUC 997
L. shannonii K769	SHAN 769
AUGMENTS	
L. esculenta K950§	ESCU 950
L. greggii K857§	GREG 857
L. lanceolata K952§	LANC 952
L. macrophylla K836§	MACR 836
L. multicapitula K880§	MULT 880
L. salvadorensis K746§	SALV 746
L. shannonii K969§	SHAN 969

†*Leucaena* spp. selections.
‡K numbers based on University of Hawaii numbering system.
§*Leucaena* spp. selections replicated once or twice.

with *Leucaena* rhizobium (TAL 1145, NiFTAL Project, Univ. of Hawaii) and planted March 20, 1991 into 5 cm dia. peat pots containing commercial planting medium (75 % peat, 25 % perlite). Seedlings were watered daily in the greenhouse until ready for transplanting. Seedlings were transplanted 2 May 1991 into an Isohyperthermic Vertic Haplustoll that had been cultivated into a fine seed bed and which had a history of corn production. Twenty seedlings $plot^{-1}$ were planted in single rows 1 m apart and 0.2 m within the row producing a plant population of 50,000 plants ha^{-1}. This design was initially intended to determine early seedling vigor (Sorensson *et al.*, 1994), references to total and forage biomass production in this paper must be intrepreted with caution since the plot layout is based on single rows which could confound biomass production due to border effects bvoth above and below ground. Irrigation was applied 2 to 3 times wk^{-1} until the end of July, but none thereafter. Seedlings were sprayed twice prior to the first harvest with Sevin (0.8 ml a.i. Carbaryl) to reduce psyllid populations. Weeding was done by hand-hoeing intrarow, and by mowing interrow.

The experimental design was an augmented randomized complete block (Brewbaker, 1994a) with 22 selections replicated four times, two selections replicated thrice (K450 and K819), six selections replicated twice and a single entry present once. Selections replicated three or

four times were analyzed together using Proc GLM in SAS. The remaining selections were considered augments and not used in the analysis of variance. A total of 5 harvests were taken over 13 months. Harvests were taken on August 14 and October 15, 1991 and on January 14, March 27 and June 2, 1992 when plant heights averaged 2.5 m in the first harvest, and 2.0 m in later harvests. Eight plants were harvested within each plot and cut at 0.25 m above ground in the first harvest and at 0.5 m in subsequent harvests. Harvested material was weighed, then subsampled and sealed in plastic bags. Subsamples were hand separated into forage (leaf and attached stem < 6 mm dia.) and wood fractions. Fractions of the subsample were weighed and then dried at 60° C for 2 weeks for DM determination.

Data were separated into 2 groups based on parentage due to significant difference ($P<0.001$) of the error variance of the replicated treatments using Proc TTEST in SAS. High yielding Group 1 was composed of all *L. pallida* species and hybrids. Group 2 consisted of the remaining 14 selections. Augments were not grouped nor were they analyzed in the analysis of variance.

Forage DM, total DM and forage percent data were analyzed as a split-plot with *Leucaena* selection as main plot and harvest date as subplot. If a selection by harvest interaction, and Bartlett's test of homogenity using chi-square analysis were significant, data were sorted by

harvest and analyzed as an RCB. Analysis of variance of each group was then tested with the appropriate error variances (Gomez and Gomez, 1984). Mean separations were performed within each group of the replicated selections only using the least significant difference method (LSD, $P<0.05$).

Psyllid damage ratings were taken on October 5, 1991 and June 1, 1992, prior to harvests 2 and 5, respectively. Six sample plants $plot^{-1}$ were rated for psyllid damage. An empirical scale of 1 to 9 (1=undamaged, 9=highest) as previously described in the literature (Wheeler, 1988) was used to rate psyllid damage on newly emerged leaf tips.

A square root transformation was used to correct for a non-normally distributed rating scale (Steele and Torrie, 1980). Non-transformed means are reported in Table 2.6 to facilitate presentation of results.

Correlation coefficients were determined for harvests 2 and 5 between forage DM, total DM, and percent forage with psyllid damage with all replicated varieties. Separate correlations were determined for forage DM and total DM yields from harvest 1 and total combined yields to seedling height measured at 42 and 84 days after transplanting. A total of 19 selections were used for the later correlations (9 in Group 1 and 10 in Group 2) as described by Sorensson *et al.* (1994).

RESULTS

Biomass yields

The highest yielding variety, K748 x K636 (PxL 2) produced 34 Mg ha^{-1} forage DM and 63.6 Mg ha^{-1} total DM (Table 2.3). Group 1 varieties averaged 22.2 Mg ha^{-1} DM forage from a total DM production of 40.3 Mg ha^{-1} (Table 2.2). Group 2 varieties produced an average of 9.3 Mg ha^{-1} forage DM and a total DM of 15.6 Mg ha^{-1} (Table 2.3). The *L. leucocephala* selections (K636, K500, and K997) in Group 2 averaged 4.2 Mg ha^{-1} forage DM and 6.7 Mg ha^{-1} total DM (Table 2.3).

Group 1 had significantly lower ($P<0.05$) percent forage fractions (57 %) when combined over all 5 harvests, while the average of varieties in Group 2 was 62 % (Tables 2.2 and 2.3). The arboreal *L. pallida* K804 had the lowest percent forage fraction 49 % among all selections.

The large range in yield and significant differences in error variances of *L. pallida* vs. the remaining selections for forage and total DM yields required that the data be seperated into groups. A significant variety by harvest date interaction for forage DM, total DM and percent forage was detected. In addition, siginificantly different ($P<0.001$) error variances at each harvest required that the data be analyzed separately by harvest.

Forage DM was significantly different ($P<0.01$) for selections in both Group I and II (Tables 2.4 and 2.5).

Table 2.2. Biomass yields of forage and total DM (Mg ha^{-1}) and percent forage for Group I *Leucaena* varieties over 13 months.

	----- Harvest # -----							
Variety	1	2	3	4	5	Total† For.	Total‡ DM	% For.§
PxL 2	4.6	8.2	5.9	6.7	8.6	34.0	63.6	53
PxL 1	5.3	5.8	4.6	4.5	5.9	26.2	48.1	54
PALL HYB	3.8	6.4	4.7	4.6	6.6	26.0	47.7	54
(DxL)xP	4.8	4.2	4.5	5.6	6.6	25.5	45.2	56
PALL 804	3.8	4.9	3.6	3.1	6.6	22.0	44.6	49
PALL 376	3.2	6.6	4.5	4.5	6.4	25.2	44.2	57
PALL 819¶	3.9	5.5	4.9	3.8	5.7	23.7	41.7	57
(DxP)xL	2.5	3.0	4.0	4.2	7.4	21.1	34.3	61
PALL 953	3.0	1.8	1.3	2.1	5.2	13.5	24.8	54
PxD	1.2	0.6	0.7	2.0	0.7	5.1	8.2	61
MEAN	3.6	4.7	3.9	4.1	6.0	22.2	40.3	57
CV	42	55	47	47	41	50	52	20
$LSD_{0.05}$	1.4	2.0	1.6	2.0	2.2	6.4	11.9	3.5

†Total forage fraction = leaves and small stems < 6 mm diameter for all 5 harvests.
‡Total DM biomass is the forage and wood fraction stems > 6 mm diameter combined.
§Percent forage is the forage DM/total DM.
¶All varieties replicated 4 times except K819 which was replicated three times.

Table 2.3. Biomass yields of forage and total DM (Mg ha^{-1}) and percent forage for Group II *Leucaena* varieties, and augmented selections over 13 months.

Variety	Harvest # 1	2	3	4	5	Total† For.	Total‡ DM	% For.§
COLL 905	1.7	3.5	3.1	1.7	5.3	15.4	25.6	60
COLL 450¶	0.6	3.2	2.7	3.2	4.7	14.4	24.9	58
DIV2 749	1.6	3.7	3.6	2.0	2.9	13.8	24.1	59
ESCU 948	4.5	2.5	1.6	1.4	4.8	14.7	23.8	63
DxL	3.2	1.4	2.4	2.0	4.5	13.5	23.7	57
LANC 393	1.7	1.4	1.9	2.0	5.4	12.4	19.5	64
SHAN 769	0.9	1.6	2.1	1.8	3.5	10.0	18.0	57
DIV2 919	1.1	1.4	1.6	2.1	3.8	10.0	17.0	58
LEUC 636	1.1	0.6	1.6	1.2	2.7	7.3	12.7	57
DIV4 156	0.9	0.4	0.9	1.4	3.5	7.2	11.0	65
DIV4 146	0.7	0.2	1.2	1.2	2.9	6.2	9.3	67
COLL 911	0.4	0.2	0.7	0.8	1.1	3.2	7.6	43
LEUC 500	1.0	0.1	0.5	0.9	1.7	3.9	5.4	73
LEUC 997	0.3	0.0	0.3	0.3	0.6	1.4	1.9	73
MEAN	1.4	1.4	1.7	1.6	3.4	9.3	15.6	62
CV	82	98	70	54	50	53	55	15
$LSD_{0.05}$#	0.6	1.0	1.1	0.8	1.2	3.2	6.1	7
AUGMENTS††								
ESCU 950	1.4	2.7	1.3	1.8	2.3	9.5	17.0	56
GREG 857	0.7	1.0	1.1	0.7	0.8	4.4	5.6	78
LANC 952	0.9	2.0	2.5	2.2	3.2	10.8	17.8	61
MACR 836	1.9	4.0	2.9	1.7	6.8	17.3	25.0	68
MULT 880	0.07	0.03	0.7	0.7	1.4	2.8	3.8	75
SALV 746	0.8	1.0	1.2	1.5	2.7	7.2	13.2	54
SHAN 969	1.1	1.4	2.1	2.1	4.5	11.3	19.2	59

†Total forage fraction = leaves and small stems < 6 mm diameter for all 5 harvests.
‡Total DM biomass is the forage and wood fraction stems > 6 mm diameter combined.
§Percent forage is the forage DM/total DM.
¶All varieties replicated four times except K450 which was replicated three times.
#LSD of replicated varieties only.
††Augments were replicated twice with the exception of K969 which was replicated once.

Table 2.4. ANOVA of GROUP I selections on an individual forage DM harvest basis with MS values from Type III sum of squares.

		MEAN SQUARES (10^{-6})				
		Forage Harvest				
Source	df	1	2	3	4	5
Block	3	3.39 *	1.89 NS	0.22 NS	3.46 NS	1.65 NS
Var.†	9	5.79***	21.34***	10.81***	8.56**	17.18***
Error	26	1.03	2.14	1.24	2.16	2.52
Total	38					

*,**,*** Significant at P<0.05, 0.01 and 0.001, respectively, NS not significant.
†Group 1 selections were PxL 1, PxL 2, PALL HYB, (DxL)xP, PALL 804, PALL 376, PALL 819, (DxP)xL, PALL 953, PxD.

Table 2.5. ANOVA of GROUP II selections on an individual forage DM harvest basis with MS values from TYPE III sum of squares.

		MEAN SQUARES (10^{-6})				
		Forage Harvest				
Source	df	1	2	3	4	5
Block	3	0.93 **	0.27 NS	0.76 NS	0.21 NS	0.91 NS
Sel.†	13	5.05***	6.59***	3.91***	1.86***	2.37 **
Error	38	0.15	0.46	0.64	0.34	0.71
Total	54					

,* Significant at P<0.05, 0.01 and 0.001, respectively, NS not significant.
†Group 2 selections were COLL 450, COLL 905, COLL 911, DIV2 749, DIV2 919, DIV4 146, DIV4 156, DxL, ESCU 948, LANC 393, LEUC 500, LEUC 636, LEUC 997, SHAN 769.

Forage DM yield of 19 varieties from the first harvest was positively correlated ($r=0.83$, $P<0.001$) with seedling height after 42 days and again ($r=0.88$, $P<0.001$) after 84 days after transplanting. Combined forage DM yields over all five harvests was also correlated with height ($r=0.66$, $P<0.001$) after 42 and ($r=0.77$, $P<0.001$) 84 days, respectively. Forage DM yields of the *Leucaena* group I and group II selections at each harvest were not correlated with GDD and solar radiation. Data for these correlations are not presented.

Psyllid resistance

Psyllid damage ratings were not grouped because the varainces of all the varities were homogenous. The lowest psyllid damage ratings for the October 5 observation period were for *L. diversifolia* K749, *L. pallida* K376, *L. esculenta* K950, and *L. pallida* x *L. diversifolia* (DxL) K806 x K156 (Table 2.6). Selection K997 had the highest psyllid damage in the first observation period ($P<0.05$), while all three *L. leucocephala* selections exhibited the highest damage ratings for the second observational period ($P<0.05$). All *L. pallida* species and hybrids showed excellent resistance to the pest with the exception of K953 (Table 2.6).

Forage DM yield and psyllid damage were negatively correlated at harvest 2 ($r = -0.59$, $P<0.001$, $n = 94$), and again at harvest 5 ($r = -0.56$, $P<0.001$, $n = 94$).

Table 2.6. Psyllid damage ratings of thirty-one *Leucaena* varieties at Waimanalo, Hawaii.

	Damage†	
Selection‡	5 Oct. 1991	1 June 1992
PxL 1	3.09 de§	1.21 a
PxL 2	2.75 cd	1.14 a
PALL HYB	2.35 bc	1.00 a
(DxL)xP	3.70 ef	1.41 ab
PALL 804	3.09 de	1.00 a
PALL 376	1.89 ab	1.00 a
PALL 819	2.24 bc	2.31 efg
(DxP)xL	4.87 h	1.29 ab
PALL 953	5.37 hij	2.15 def
PxD	2.09 ab	1.00 a
COLL 905	2.18 bc	1.14 a
COLL 450	2.26 bc	2.18 defg
DIV2 749	1.65 a	1.00 a
ESCU 948	2.08 ab	1.00 a
DxL	5.82 ij	2.78 ghi
LANC 393	4.63 gh	2.55 fgh
SHAN 769	4.07 g	1.78 bcde
DIV2 919	3.81 f	1.21 a
LEUC 636	5.06 hi	5.00 j
DIV4 156	5.08 hi	1.92 cde
DIV4 146	5.12 hi	1.72 bcd
COLL 911	4.67 gh	1.07 a
LEUC 500	5.93 ij	4.91 j
LEUC 997	6.13 j	4.91 j
ESCU 950	2.68 cd	1.00 a
GREG 857	2.39 bc	1.29 ab
LANC 952	2.27 bc	1.00 a
MACR 836	3.74 ef	1.45 abc
MULT 880	5.27 hij	3.00 hi
SALV 746	5.18 hi	3.21 i
SHAN 969	n/a¶	n/a
Average	3.65	1.82

†Damage based on a scale of 1-9 with 1=no damage and 9=complete defoliation.
‡*Leucaena* selections from table 1.
§Means within columns followed by the same letter are not different ($P<0.05$).
¶Entry replicated one time, no psyllid data taken.

DISCUSSION

The experimental design of this experiement was initially set up to test early seedling vigor (Sorensson *et al.*, 1994). The single row plots in this trial amplified inter-specific competition both above and below ground. Therefore yields reported herein must be viewed with caution.

The higher yields of Group I are due to increased psyllid resistance and superior seedling vigor (Sorensson *et al.*, 1994). Forage DM yields of the best Group I varieties are similar to pre-psyllid infestation yields for *L. leucocephala* in Hawaii (Brewbaker *et al.*, 1972) at 30 Mg ha^{-1}, and are higher than 12.3 Mg ha^{-1} under intensive harvest management on Hawaii (Guevarra *et al.*, 1978). The average forage DM yield of group I selections is approximately three times higher than *L. leucocephala* K636, and the two best yielding hybrids PxL 1 and PxL 2 produced approximately four times more forage than K636. Group I forage yields were higher than those reported in Florida for the best *L. leucocephala* genotypes at 14 to 16 Mg ha^{-1} (Austin *et al.*, 1995a; Othman *et al.*, 1985). However, it must be noted that optimum growing conditions for *Leucaena* spp. in north-central Florida are restricted due to sub-freezing ($0\,^{0}C$) winter temperatures.

The highly positive correlations between yield and seedling vigor suggests that two main breeding objectives

can be attained through hybridization with *L. pallida*. *Leucaena leucocephala* seedlings are notoriously slow starters (Lesleighter and Shelton, 1986), and the hybridization of this species with *L. pallida* not only improves psyllid resistance but also seedling vigor.

A significant variety by harvest interaction is the result of growth habit differences of plants prior to first harvest i.e. *L. pallida* species and hybrids had greater seedling vigor than the other selections (Sorensson *et al.*, 1994) based on plant height and dry matter weight. The fluctuation of psyllid populations and the pressure exerted by these pests also contributed to varying forage production over the separate harvests.

Non-significant correlation between forage DM, GDD and mean monthly solar radiation may be due to the short duration of the experiment (Appendix B), and contradict earlier findings from Hawaii (Evensen, 1985; Guevarra *et al.*, 1978). Both Evensen (1985) and Guevarra *et al.* (1978) irrigated their *Leucaena* throughout the trial, while in this study irrigation was stopped prior to the first harvest. Total DM yield in harvest 1 was lower than harvest 2 although solar radiation was lower during the time period leading up to harvest 2. The absence of irrigation in later harvests probably confounded the effect of light and temperature on harvests 2-5 since optimum soil moisture conditions were no longer controlled. Total winter solar

radiation in Waimanalo is approximately one-half that of summer, while the difference in average temperature is about 3^0 C (Appendix A). The lack of correlation between these parameters suggests that the majority of variation in forage DM yield in this experiment is attributed to psyllid resistance and seedling vigor.

The large range in percent forage DM (Tables 2.2 and 2.3) is the result of variation in plant height at harvest. Psyllid susceptible selections like *L. leucocephala* K997 and K500 produced very little biomass over all harvests. The stunted condition of these plants resulted in greater forage DM ratios despite increased damage to the leaves since the forage DM forage fraction encompasses not only leaves but also small stems.

In Hawaii, psyllids infest *Leucaena* continuously, even though populations fluctuate significantly during the year (Wheeler and Brewbaker, 1989). Overall, psyllid infestation was higher for the period preceding harvest 2 than the period prior to harvest 5. Psyllid populations are never stable (Austin *et al.*, 1995b; NFTA, 1988) which explains why damage scores were lower at harvest 5 compared to harvest 2. *Leucaena pallida* varieties, with the exception of K953, exhibited excellent psyllid resistance at both observations. Interesting to note are the low psyllid damage ratings of *L. diversifolia* K749, and *L. collinsii* selections K905 and

K450. Both are Group II selections indicating that psyllid resistance alone is not enough to produce top yields.

Negative correlations between forage DM yield and psyllid damage indicates the need to plant resistant varieties in areas with year-round psyllid pressure. This finding is in contrast to those of Florida where negetive correlations between edible and total DM and psyllid damage (Austin *et al.*, 1995b) were significant in only 2 of 11 harvests during a 3 year period.

CONCLUSIONS

Interspecific hybrids of *L. pallida* x *L. leucocephala* produced superior forage DM yields in this trial. Excellent psyllid resistance, and early seedling vigor contributed by *L. pallida* are vital for successful forage or biomass operations in lowland Hawaii. However, high psyllid resistance did not always result in high biomass yields as evidenced by K749. Breeding programs can incorporate psyllid resistance with high forage and total DM yields using *L. pallida* as one parent in a hybrid cross.

CHAPTER 3

LEUCAENA FORAGE DRY MATTER PRODUCTION, PSYLLID RESISTANCE, DRY MATTER DIGESTIBILITY, AND TANNIN CONCENTRATION AT TWO ENVIRONMENTS IN HAWAII

ABSTRACT

While earlier research conducted at Waimanalo, Hawaii, indicated superior forage yields of *Leucaena* F_1 hybrids, it is necessary to test these hybrids and suitable *Leucaena* species in different environments to determine one or two top yielding selections for Hawaii and to determine if tannin concentration, digestibility, and psyllid resistance were correlated to forage quality. Nineteen *Leucaena* spp. selections including *L. pallida* K376, K748, K806, K953 and the intraspecific hybrid K806xK748; *L. leucocephala* K636; *L. diversifolia* K156, K749, K784 and K785; *L. esculenta* K948; F_1 hybrids *L. pallida* x *L. leucocephala* K748xK636, K748xK584, K806xK636, K806xK584, K804xK636 and (K156xK636)xK806; and advanced hybrids *L. pallida* x *L. leucocephala* KX2 F_4 (K376xK8) and *L.diversifolia* x *L.leucocephala* KX3 F_3 (K156xK636) were investigated at two locations in Hawaii. The low elevation site was at the Waimanalo Research Station (WAI) and high elevation (835 m) was at the Mealani Research Station (MEA). Parameters measured include total and forage dry matter (DM) yields,

psyllid damage, digestibility (IVDMD) and total condensed tannin (TANN) concentrations. The best yielding varieties at both locations in yr one were the F_1 hybrids K748xK636, and K748xK584. At WAI, K748xK636 produced 35.0 Mg ha^{-1} total DM biomass from which 16.7 Mg ha^{-1} DM forage was produced after five harvests. At MEA, K748xK636 produced 10.0 Mg ha^{-1} total DM biomass from which 4.4 Mg ha^{-1} DM forage was produced after two harvests. Psyllid populations in WAI fluctuated tremendously in yr one and plant resistance varied. The most susceptible selection ($P<0.001$) was *L. leucocephala* K636 and the most resistant selections were *L. diversifolia* (2n) K749 and *L. esculenta* K948. Psyllid damage was inversely correlated with forage DM at WAI ($r=-0.37$, $P<0.05$) only once in yr 1. IVDMD was different ($P<0.01$) between locations with the highest digestibility observed from forage DM at WAI. IVDMD ranged from 598 to 776 g kg^{-1} at WAI to 488 to 673 g kg^{-1} at MEA. Overall, K636 had the best average IVDMD with 700 g kg^{-1} while *L. diversifolia* K784 was 600 g kg^{-1}. TANN concentrations ranged from 96 to 118 mg g^{-1} at WAI and 67 to 122 mg g^{-1} at MEA. TANN concentration of K636 was lower at WAI (96 mg g^{-1}) than MEA (116 mg g^{-1}). Higher TANN of K636 at MEA is probably the result of increased phenolic production in response to environmental stress. TANN were correlated once positively and once inversely to forage DM twice ($r=0.36$, $P<0.05$ and $r=-0.43$, $P<0.05$). Psyllid damage and TANN were correlated

once ($r=0.32$, $P<0.05$), while IVDMD and TANN were correlated at MEA ($r=0.43$, $P<0.01$). The success of the F_1 hybrids in distinctly different environments represents a new direction for the *Leucaena* breeding program. It must now concentrate on developing two or three top lines for multiple environments in Hawaii and elsewhere.

INTRODUCTION

There is a need to improve the nutritional quality of grass pastures in order to increase beef and dairy cattle productivity in Hawaii. Hawaii's 1992 inventory of all cattle and calves (beef and dairy) was 178,000 head (Hawaii Agricultural Statistics Service, 1993). Total cash receipts from the sale of cattle and calves for 1992 was $29.2 million (Hawaii Agricultural Statistics Service, 1993). A large proportion of the land used for livestock production in Hawaii is located in sub-tropical, high rainfall areas (Sherrod and Ishizaki, 1966). Most improved pastures in Hawaii consist of kikuyu grass (*Pennisetum clandestinum*) and pangola grass (*Digitaria decumbens*) in swards devoid of legumes (Sherrod and Ishizaki, 1966). Incorporating legumes into grass pastures should increase the pastures' feeding value (Sollenberger *et al.*, 1987) and decrease dependence on N fertilizer (Evers, 1985).

The legume *Leucaena* is a multipurpose tree crop native to Mexico and Central America (Brewbaker, 1987a). *Leucaena*

leucocephala (Lam.) De Wit is the most commonly grown species of *Leucaena* in the world (Brewbaker *et al.*, 1989) and is found growing wild throughout the Hawaiian islands (Kinch and Ripperton, 1962; Merlin, 1976). *Leucaena* is a persistent, warm-season, perennial legume tree that has a history of forage use in the Hawaiian Islands (Takahashi and Ripperton, 1949). Later reports indicate DM forage biomass yields approaching 4 Mg ha^{-1} $harvest^{-1}$ (Kinch and Ripperton, 1962). Assuming 4 to 5 harvests $year^{-1}$ in lowland Hawaii forage production of approximately 16 to 20 Mg ha^{-1} yr^{-1} is possible (Austin, Chapter 2). Brewbaker *et al.* (1972) reported DM forage yields of the three best *L. leucocephala* varieties K8, K28 and K67 at 30 Mg ha^{-1} yr^{-1} on Oahu and Kauai, while Guevarra *et al.* (1978) reported total DM biomass production of K8 at 21 Mg ha^{-1} yr^{-1} (12.3 Mg ha^{-1} yr^{-1} forage DM) on the island of Hawaii.

Forage quality varies among the 16 *Leucaena* species. Digestibility (IVOMD) ranged from 39 to 57 % for eight *Leucaena* species and hybrids in Florida (Austin *et al.*, 1995a). Leaf IVDMD of 59 different *Leucaena* species and hybrids tested in Hawaii ranged from 53 to 85 % (Sorensson, 1993). Pinnule fractions in his study were more digestible, averaging 63 %, than twigs, which averaged 55 %. The midribs and rachii had even lower digestibility at 45 % (Sorensson, 1993). Tannins are thought to affect feed intake, reduce palatability (Barry and Duncan, 1984), and

increase by-pass protein levels (Butler, 1989). Castillo, (1994) reported leaf plus stem tannin concentrations of 16 *Leucaena* species and hybrids ranging from 54 mg g^{-1} for *L. pallida* K953 to 141 mg g^{-1} for *L. diversifolia* K749. Constantinides and Fownes (1994) reported *L. leucocephala* leaves to have a total tannin concentration of 90 mg g^{-1} in Hawaii.

Psyllids (*Heteropsylla cubana* Crawford) are insect pests of *Leucaena* native to Mexico and Central America. They arrived in Hawaii in 1984 causing extensive damage to susceptible *Leucaena* stands (Nakahara and Lai, 1984). Psyllids are members of the order Homoptera and family Psyllidae (Beardsley, 1986). Psyllids infest the young, actively growing tips of the plant, feeding and ovipositing in these areas (Beardsley, 1986). The most commonly grown species, *L. leucocephala*, is highly susceptible to psyllid damage (Brewbaker *et al.*, 1989). When newly arrived, psyllids cause extensive damage in areas devoid of natural enemies (Nakahara and Funasaki, 1986). However, infestations level off after about the second season of growth (Van Den Beldt and Napompeth, 1992) if *Leucaena* is managed for wood production.

The abundance of young leaf material associated with forage management provides an optimal environment for promoting continuous psyllid infestations. Psyllid populations fluctuate tremendously in Hawaii with peak

infestations occurring in the rainy seasons between November to March (Wheeler, 1988; M.T. Austin, 1994, personal observation). In an earlier experiment in Hawaii psyllid damage and biomass yield were inversely correlated (Austin, Chapter 2). Varieties with the highest resistance and yield were all *L. pallida* species and hybrids (Austin, Chapter 2).

There are 15 to 16 recognized species within the genus *Leucaena* (Sorensson and Brewbaker, 1994). Variability for psyllid resistance in the genus is extensive (Brewbaker *et al.*, 1989). Resistant species include *L. pallida*, *L. esculenta* and *L. collinsii* (Sorensson and Brewbaker, 1986). The tetraploid *L. pallida* is best suited to developing fertile hybrids with *L. leucocephala*. *Leucaena pallida* is thought to be an amphidiploid between *L. esculenta* n=26 x *L. diversifolia* ssp. *trichandra* n=26 (Pan, 1985; Pan and Brewbaker, 1988). Both *L. pallida* and *L. esculenta* exhibit excellent psyllid resistance over several environments (Austin, Chapter 2; Austin *et al.*, 1995b).

The purpose of this study was to determine if the F_1 hybrids evaluated as superior from an earlier experiemnt can produce well across diverse environments. Environmental constraints include psyllid resistance in the lowlands and cool temperature tolerance at higher elevations. An important objective was to determine if forage quality as a function of high digestibility and psyllid resistance is affected by total phenol concentrations.

MATERIALS AND METHODS

Two locations in Hawaii were selected to test superior materials identified in an earlier *Leucaena* forage trial conducted during 1991-1992. The Waimanalo Research Station (WAI) on Oahu lies at sea level with an average rainfall of 1380 mm yr^{-1} and temperatures ranging from 22-27 °C. Soil is an Isohyperthermic Vertic Haplustoll consisting of basaltic colluvium underlaid by coral with a soil pH of approximately 6.0 to 6.5. The Mealani Research Station (MEA) on Hawaii is 853 m above sea level with an average rainfall of 1500 mm yr^{-1} and temperatures ranging from 11-23 °C. Soil is a Thixotropic Isomesic Hydric Dystrandept and is derived from volcanic ash with a pH ranging from 5.5 to 6.5.

A total of 19 entries from 4 species, including 9 hybrids, were tested in this experiment (Table 3.1). Seeds were scarified using boiling water and then soaked overnight. Seeds were inoculated with Leucaena *rhizobium* (NIFTAL, Univ. of Hawaii) and planted 15 February 1993 into plastic dibble tubes filled with peat and vermiculite in the greenhouse. Seedlings were watered three times daily.

Seedlings were transplanted 26 May 1993 at MEA and 3 June 1993 at WAI. At MEA seedlings were transplanted onto land that had previously been planted to trees. At WAI seedlings were transplanted into fallow land. No irrigation was used at either site.

Table 3.1. List of *Leucaena* selections planted at two locations, Waimanalo and Mealani, Hawaii.

Replicated selections
K636 *L. leucocephala* (CONTROL)
3WAY HYBRID (K156 x K636) x K806
KX3 SC.† F_3 *L. diversifolia* x *L. leucocephala*
KX2 SC.† F_4 *L. pallida* x *L. leucocephala*
K748XK806 F_1 *L. pallida*
K748 OP *L. pallida*
K806 OP *L. pallida*
K748XK636 F_1 *L. pallida* x *L. leucocephala*
K806XK636 F_1 *L. pallida* x *L. leucocephala*
K748XK584 F_1 *L. pallida* x *L. leucocephala*
K156 *L. diversifolia* 4x
K749 *L. diversifolia* 2x
K784 *L. diversifolia* 4x
Non-replicated
K804 x K636 F_1 *L. pallida* x *L. leucocephala*
K806 x K584 F_1 *L. pallida* x *L. leucocephala*
K785 *L. diversifolia* 4x
K953 *L. pallida*
K376 *L. pallida*
K948 *L. esculenta*

†SC stands for special composite made from advanced KX2 and KX3 parent trees selected for higher psyllid resistance and vigor at Waimanalo.

The experimental design was an augmented randomized complete block. Thirteen entries were replicated 3 times at both sites, 2 selections were replicated once at each location, and the remaining four selections replicated once at only one location. Seedlings were planted in double rows one meter apart and 25 cm apart. Plots were separated by 1.5 m producing a plant population of 35,500 plants ha^{-1}.

Plants were harvested five times for biomass at WAI. Harvest dates were 28 Oct. 1993, 2 Feb., 5 May, 7 July, and 9 Sept. 1994. Two harvests were taken at MEA in the first year on 20 Dec. 1993 and 30 June 1994. Plant heights averaged 2 m at harvest. A total of eight plants were harvested, four from each side of the double row. A meter of row was cut at 0.5 m above ground level. Materials were weighed in the field, and a randomly selected subsample (1 to 2 cut stems) were removed and hand-separated into forage (leaf and small stem > 6mm) and woody fractions (stems > 6 mm diameter). Fractions were weighed wet and then dried at 60 ^{o}C for 2 wk. Dried forage fractions were ground in a Thomas-Wiley mill to 1mm diam.

In-vitro digestibility IVDMD was analyzed for the first harvest at both locations. IVDMD was measured from 1 mm ground samples using a 48 hour rumen digestion followed by treatment by neutral detergent for 1 hour as described by Goering and Van Soest (1970).

Tannins were analyzed for the first three harvests from WAI and first harvest at MEA (representing year one), using a modified form of Quarmby and Allen (1989) procedure. Soluble polyphenols were extracted in hot 50% aqueous methanol and centrifuged. A Folin-Ciocalteu reagent was used and samples analyzed using a spectrophotometer.

Psyllid damage ratings were taken monthly only at WAI since psyllids are not a threat at higher elevations. Leaf tips of 4 plants per plot (12 plants in total) were examined for plant damage using an empirical scale of 1 to 9 as described by Glover (1987), where 1= no damage, and 9= complete defoliation. Psyllid data were square root transformed to correct for a non-normally distributed rating scale (Steele and Torrie, 1980). If the variances were non-normally distributed after transformation then data were analyzed using Proc NPAR1WAY (SAS, 1986) using Wilcoxon scores, a method of ranking the data that does not require a normal distribution to determine treatment differences. Mean separations were conducted on the ranked data using Kruskal-Wallis chi square in SAS.

Total DM, forage DM, IVDMD, and TANN averages were analyzed as a split-plot with location as the main plot and selection as the sub-plot. If a significant location by selection interaction was detected, data were separated by location and reanalyzed. The five harvests in Waimanalo were analyzed as a strip-block with harvest as the main plot

and selection as the sub-plot and harvestxselection as the sub-sub-plot. If a treatment by harvest interaction was significant or if harvests were different, data were then analyzed separately by harvest and analyzed as a RCB. IVDMD was not transformed since the range of these data did not exceed 40 % (Little and Hills, 1978). Total DM, forage DM, TANN and IVDMD were analyzed by single degree of freedom contrasts using Proc GLM in SAS (1986). Pearson correlation coefficients (SAS, 1986) were determined for each date for total DM, forage DM, psyllid damage, IVDMD and TANN.

RESULTS

Biomass yields

The best yielding selections at both locations were the F_1 hybrids K748 x K636 and K748 x K584. Total DM of the replicated selections at each location ranged in yield from 8.9 to 35.0 Mg ha^{-1} in WAI (Table 3.2) to 0.2 to 10.7 Mg ha^{-1} at MEA (Table 3.3). Forage DM ranged from 4.4 to 16.7 at WAI (Table 3.2) to 0.16 to 4.4 Mg ha^{-1} at MEA (Table 3.3). The control K636 had lower ($P<0.05$) total DM yield than all other selections at harvests 3 and 4 in WAI (Table 3.2) and for both harvests ($P<0.001$) at MEA (Table 3.3). Total DM yield for K636 at MEA was 0.2 Mg ha^{-1} (Table 3.3). *Leucaena*

Table 3.2. Forage and total biomass yields Mg ha^{-1} with LSD's for five harvests at Waimanalo, Hawaii.

	---Forage Harvest†---							
Selection	1	2	3	4	5	Total‡ Forage	Total§ Biomass	%¶ Forage
Replicated								
K748xK636 F_1	2.5	4.8	5.4	2.7	1.3	16.7	35.0	48
K748xK584 F_1	2.1	3.7	2.9	3.3	2.5	14.5	32.9	44
K806 OP	3.0	3.0	2.8	2.7	1.8	13.3	28.8	46
K806xK636 F_1	2.5	3.3	3.3	2.9	2.1	14.2	29.4	48
K748xK806 F_1#	1.7	2.6	3.2	1.5	2.7	11.7	24.4	48
K748 OP	2.0	2.5	2.4	3.9	1.3	12.1	27.8	44
KX3 SC	1.9	2.3	3.1	1.7	1.1	10.2	19.5	52
KX2 SC	1.4	2.6	2.9	2.3	1.2	10.4	20.4	51
3way	1.4	2.1	2.3	1.9	0.9	8.6	18.2	47
K784	1.1	1.9	2.6	1.1	1.0	7.7	14.0	55
K636	1.4	2.4	1.5	1.1	1.8	8.2	14.7	56
K749	0.7	1.0	1.5	0.8	0.4	4.4	8.9	49
K156	0.8	1.3	1.0	1.8	0.7	5.7	10.6	54
AVG††	1.7	2.6	2.7	2.1	1.4	10.8	21.9	49
$LSD_{0.05}$‡‡	1.0	1.3	1.8	1.5	1.6	1.6	3.4	14
Non-replicated								
K804xK636 F_1	2.4	2.8	2.7	1.6	1.1	10.6	24.3	44
K376	2.4	2.7	2.8	2.2	1.2	11.3	22.2	51
K785	0.6	1.6	1.7	1.5	0.0	5.4	9.7	56
K948	0.0	0.6	1.2	1.0	0.4	3.2	5.5	58

†Harvests occurred on 28 Oct. 1993, 2 Feb. 5 May, 7 July, and 9 Sept. 1994.
‡Total Forage DM is the leaves and small stems < 6 mm diameter.
§Total Biomass DM is Forage DM and stems > 6 mm diameter.
¶% Forage is (Forage DM/Total DM) x 100.
#K748xK806 replicated only twice at Waimanalo, all other selections replicated three times.
††Average of the replicated selections only.
‡‡LSD's of replicated selections only.

Table 3.3. Forage and total biomass yields Mg ha^{-1} with LSD's for two harvests at Mealani, Hawaii.

Selection	Forage Harvest 1	2	Total† Forage	Total‡ Biomass	%§ Forage
Replicated					
K748xK636 F_1	2.4	2.0	4.4	10.0	44
K748xK584 F_1	1.6	2.1	3.7	10.7	35
K749	1.6	2.3	3.9	9.0	43
3way	1.8	1.1	2.9	6.6	44
KX3 SC	1.9	1.8	3.7	7.2	51
K806 OP	1.9	1.1	3.0	5.7	53
K748xK806 F_1	1.2	1.3	2.5	6.2	40
K748 OP	1.3	1.1	2.4	5.6	43
K784	1.5	1.6	3.1	5.8	53
K156	1.4	0.9	2.3	4.7	49
K806xK636 F_1	0.8	1.3	2.1	3.7	57
KX2 SC	0.8	1.1	1.9	3.8	50
K636	0.06	0.1	0.16	0.2	80
AVG.¶	1.4	1.4	2.8	6.1	49
$LSD_{0.05}$#	0.9	0.7	0.8	1.9	22
Non-replicated					
K953	1.5	1.2	2.7	7.3	37
K785	1.6	0.6	2.2	4.8	46
K804xK636 F_1	1.3	1.3	2.6	6.1	43
K806xK584 F_1	1.3	2.7	3.0	6.6	45

†Total Forage DM is the leaves and small stems < 6 mm diameter.
‡Total Biomass DM is Forage DM and stems > 6 mm diameter.
§% Forage is (Forage DM/Total DM) x 100.
¶Average yield of replicated selections only.
#LSD's of replicated selections only.

Table 3.4. Single degree of freedom contrasts for total DM, forage DM, tannins and IVDMD at Waimanalo and Mealani from 1993 to 1994.

	Waimanalo					Mealani	
	Harvests						
	1	2	3	4	5	1	2
Total DM							
K636 vs. ALL†	NS	NS	*	**	NS	***	***
DIV4 vs. PALL & ADVHYB	**	***	***	***	*	NS	NS
K749 vs. K156 & K784	NS	NS	NS	NS	NS	NS	NS
K156 vs. K784	NS	NS	NS	NS	NS	NS	NS
PALL vs. ADVHYB‡	*	*	NS	*	*	NS	NS
PALL sp. vs. PALL F_1	NS	NS	NS	NS	NS	NS	NS
K806 vs. K748,K748xK806	*	NS	NS	NS	NS	NS	NS
K748 vs. K748xK806	NS	NS	NS	**	NS	NS	NS
3way vs. ALL Pall F_1	*	**	*	*	*	NS	NS
K748xK584 vs. K806xK636 & K748xK636	NS	NS	NS	NS	NS	*	*
K806xK636 vs. K748xK636	NS	**	NS	NS	NS	**	*
KX2sc vs. KX3 sc	NS	NS	NS	NS	NS	NS	NS
Forage DM							
K636 vs. ALL	NS	NS	NS	**	*	***	***
DIVS vs. PALL & ADVHYB	***	***	**	***	NS	NS	*
K749 vs. K156 & K784	NS	NS	NS	NS	NS	NS	NS
K156 vs. K784	NS	NS	NS	NS	NS	NS	NS
PALL vs. ADVHYB‡	NS	NS	NS	NS	NS	NS	NS
PALL sp. vs. PALL F_1's	NS	*	NS	NS	NS	NS	NS
K806 vs. K748,K748xK806	*	NS	NS	NS	NS	NS	NS
K748 vs. K748xK806	NS	NS	NS	**	NS	NS	NS
3way vs. ALL Pall F_1's	*	***	*	*	NS	NS	NS
K748xK584 vs. K806xK636 & K748xK636	NS	NS	NS	NS	NS	NS	NS
K806xK636 vs. K748xK636	NS	*	*	NS	NS	***	*
KX2sc vs. KX3 sc	NS	NS	NS	NS	NS	*	NS

Table 3.4, Continued

	Waimanalo					Mealani	
	Harvests						
	1	2	3	4	5	1	2
Tannins %							
K636 vs. ALL	**	NS	NS			NS	
DIVS vs. PALL & ADVHYB	NS	NS	NS			NS	
K749 vs. K156 & K784	NS	**	NS			***	
K156 vs. K784	*	NS	NS			NS	
PALL vs. ADVHYB‡	*	NS	NS			NS	
PALL sp. vs. PALL F_1's	NS	*	NS			NS	
K806 vs. K748,K748xK806	*	NS	NS			NS	
K748 vs. K748xK806	*	NS	NS			NS	
3way vs. ALL Pall F_1's	NS	NS	NS			NS	
K748xK584 vs. K806xK636 & K748xK636	NS	NS	NS			NS	
K806xK636 vs. K748xK636	NS	NS	NS			NS	
KX2sc vs. KX3 sc	NS	NS	NS			NS	
IVDMD %							
K636 vs. ALL	NS					*	
DIVS vs. PALL & ADVHYB	NS					NS	
K749 vs. K156 & K784	NS					**	
K156 vs. K784	NS					NS	
PALL vs. ADVHYB‡	NS					NS	
PALL sp. vs. PALL F_1's	NS					NS	
K806 vs. K748,K748xK806	NS					NS	
K748 vs. K748xK806	NS					*	
3way vs. ALL Pall F_1's	NS					NS	
K748xK584 vs. K806xK636 & K748xK636	NS					NS	
K806xK636 vs. K748xK636	NS					NS	
KX2sc vs. KX3 sc	NS					NS	

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively; NS = not significant.
†Leucaena selections: *L. leucocephala* K636, *L. pallida* K748, K806 and K748xK806, *L. pallida* hybrids K748xK636, K748xK584, K806xK636, 3Way=(K156xK636)xK806, *L. diversifolia* K749 (2n), K156 and K784 (4n), KX2sc (K376xK8 F_4) and KX3sc (K156xK636 F_3).
‡ADVHYB are the advanced hybrid selections KX2sc and KX3sc.

diversifolia K749, K156 and K784 had lower ($P<0.01$) total and forage DM yields than the combined *L. pallida* and advanced hybrids over five harvests at WAI (Tables 3.2 and 3.4) while there was no difference in yield between these groups at MEA (Tables 3.3 and 3.4). The *L. pallida* selections outyielded ($P<0.05$) the advanced progeny KX2 and KX3 selections in total DM four times at WAI, but never in terms of forage DM (Tables 3.2 and 3.4). Among the F_1 *L. pallida* hybrids, the 3-way cross had lower total DM yields ($P<0.05$) than K806x636, K748xK636 and K748xK584 combined over all five harvests at WAI. K748xK636 produced more forage DM ($P<0.05$) than K806 x K636 twice at WAI and twice at MEA. Some selections performed well at one location but not the other. Total and forage DM production at WAI was 21.9 and 10.8 Mg ha^{-1}, respectively while at MEA these numbers were reduced to 6.1 and 2.8 Mg ha^{-1}, respectively. *Leucaena leucocephala* K636 production was affected most by cold temperatures. Total and Forage DM production was 14.7 and 8.2 Mg ha^{-1} at WAI, but only 0.2 and 0.16 Mg ha^{-1} at MEA. *L. diversifolia* K749 was also affected by temperature but in the opposite direction to that of K636. K749 produced 8.2 Mg ha^{-1} total DM at WAI (Table 3.2), but 9.0 Mg ha^{-1} total DM at MEA (Table 3.3).

A location by selection interaction ($P<0.05$) for both total and forage DM required that the data be analyzed separately by location (Table 3.5).

Table 3.5. ANOVA of thirteen *Leucaena* species and hybrids grown in two environments in Hawaii. MS values based on type III sum of squares.

Source	df	-MS Values (10^{-6})- Total† DM	Forage‡ DM	----MS Values---- TANN§	IVDMD¶
LOCATION	1	30.93*	16.82**	559.38 NS	0.181*
BLK(LOC)	4	2.76	0.49	197.36	0.013
SELECTION	12	12.79***	2.23***	457.75**	0.009*
LOCxSEL	12	5.36**	1.07***	252.91 NS	0.008 NS
ERROR b	47	1.63	0.28	143.08	0.004
TOTAL	76				

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; NS = not significant.
†Total DM = forage DM and stems > 6 mm diameter, based on average of five harvests at Wai and two harvests at MEA.
‡Forage DM = leaves and stems < 6 mm diameter, based on average of five harvests at WAI and two harvests at MEA.
§Condensed tannins mg g^{-1}, based on average from three harvests at WAI and one harvest at MEA.
¶In vitro dry matter digestibility, based on harvest one data at both WAI and MEA.

Table 3.6. ANOVA of thirteen *Leucaena* species and hybrids grown over five harvests in Waimanalo, Hawaii. MS Values based on type III sum of squares.

Source	df	-MS Values (10^{-6})- Total† DM	Forage‡ DM	Damage§
BLK	2	6.19 NS	1.45 NS	0.26 NS
HARVEST	4	47.71***	10.11***	23.96***
HARxBLK	8	2.48	0.59	0.22
SELECTION	12	43.89***	7.88***	4.26***
SELxBLK	23	6.20	1.26	0.22
HARVxSEL	48	3.88*	0.89*	1.10***
ERROR c	91	2.43	0.57	0.18
TOTAL	189			

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; NS = not significant.
†Total DM = forage DM and stems > 6 mm diameter.
‡Forage DM = leaves and stems < 6 mm diameter.
§Psyllid damage ratings were based on 1-9 scale (1=no damage,9=complete defoliation).

Data for the first five harvests at WAI were analyzed as a split-block over harvest. Harvests and harvest by selection were different ($P<0.05$) and this resulted in the data being analyzed separately by harvest (Table 3.6).

Psyllid damage

Psyllid damage was not rated at MEA since psyllids do not cause significant damage to plants at that altitude in Hawaii. Psyllid damage was analyzed separately by month at WAI since pest populations fluctuated tremendously (Figure 3.1). as a result only months where psyllid damage was 2 or higher were further analyzed.

Psyllid damage in WAI varied greatly in the first year, reaching peak damage levels in November 1993 (Figure 3.1). A non-normal distribution of psyllid damage for all monthly ratings was detected. *Leucaena* selections differed ($P<0.05$) for psyllid damage eight of the 14 months when analyzing the ranked data.

L. leucocephala K636 and *L. diversifolia* K156 had the highest overall psyllid damage ratings over the 14 month rating period. *Leucaena leucocephala* K636 had the highest psyllid damage ratings ($P<0.001$) seven out of 14 months, while *L. diversifolia* K156 was highest once (Figure 3.1).

The advanced progeny KX2 and KX3 selections had intermediate levels of resistance and did not differ between

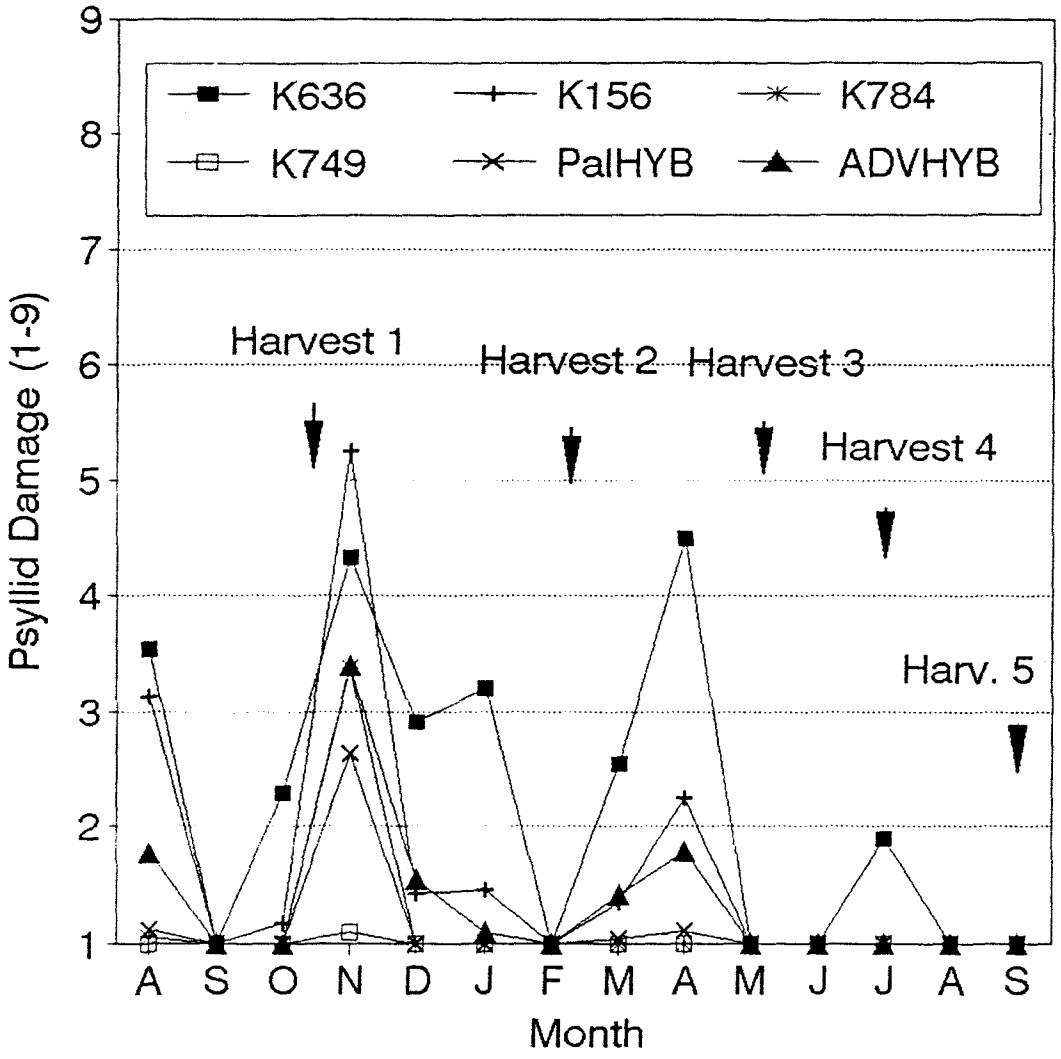

Figure 3.1. Psyllid damage of *Leucaena* spp. and hybrids at Waimanalo Research Station from 1993 to 1994.

Table 3.7. Non-parametric analysis (Kruskal-Wallis chi-square test) of psyllid damage ratings as derived from Wilcoxon Rankings at Waimanalo for 14 months from 1993 to 1994.

	Months†						
Chi-square test‡	1	3	4	5	6	8	9
K749, K784, K156	***	NS	***	*	**	*	***
K784 vs K156	***	NS	**	*	*	*	***
3way vs. K748xK636 K748xK584, K806xK636	NS	NS	NS	NS	NS	NS	NS
K748xK636 vs K748xK584 K806xK636	NS	NS	NS	NS	NS	NS	NS
K748xK636 vs K806xK584	NS	NS	NS	NS	NS	NS	NS
K748xK806 vs K806, K748	NS	NS	NS	NS	NS	NS	NS
KX2 vs KX3	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the P< 0.05, 0.01 and 0.001 probability level, NS = not significant.
†Only months where psyllid damage was 3 or higher were analyzed. Months correspond to Aug, Oct, Nov, Dec, 1993 and Jan, Mar, Apr, 1994.
‡Chi-square rankings of the various comparisons were developed using Wilcoxon Scores in Proc NPAR1WAY in SAS. Significant values indicate that these particular comparisons differed in psyllid damage.

themselves over the seven high psyllid rating periods (Table 3.7). No differences in damage were observed between any of the *L. pallida* species and hybrids including the 3-way hybrid (Table 3.7).

Within the *L. diversifolia* selections, psyllid damage levels differed ($P<0.05$) between K749, K784 and K156 six out of seven high psyllid rating periods. K749 2n and K784 4n psyllid damage ratings differed ($P<0.001$) once, K784 4n and K156 4n differed between themselves ($P<0.05$) six of the seven high psyllid rating periods (Table 3.7).

Since K636 had significantly higher psyllid damage ratings than all other selections, it is represented by a single line in Figure 3.1. The *L. pallida* species and hybrids are represented by a single line (PALHYB) since damage ratings between them did not differ over all high damage periods. The advanced hybrids KX2 and KX3 did not vary in damage and were also combined into a single line entitled ADVHYB. Differences between K784 and K156 required that a separate line be developed for each selection, while K749 was also given a separate line based on its different ploidy level from K784 (Figure 3.1).

Tannin concentrations

The average TANN concentration over both sites was 108 mg g^{-1}. Forage DM TANN at WAI ranged from 84 to 127 mg g^{-1} over three harvests. Overall, at WAI K156 had the highest

Table 3.8. Tannin concentrations in *Leucaena* forage fractions (leaves and small stems) with LSD's from harvests 1-3 at Waimanalo and harvest 1 at Mealani, Hawaii.

	Location				
	Waimanalo†			Mealani‡	MEAN§
Selection	1	2	3	1	
	mg g^{-1}, DM				
Replicated					
K748xK636 F_1	112	106	92	101	103
K748xK584 F_1	109	106	121	93	107
K749	112	84	106	67	92
3way	109	101	136	102	112
KX3 SC	120	103	107	101	108
K806 OP	121	100	108	103	108
K748xK806 F_1	123	100	115	101	110
K748 OP	102	101	106	103	103
K784	109	108	123	113	113
K156	127	117	112	123	120
K806xK636 F_1	103	98	111	107	105
KX2 SC	125	100	110	119	114
K636	95	87	107	115	101
Average¶	112	101	112	103	108
$LSD_{0.05}$#	16	23	40	26	25
Non-replicated					
K804xK636 F_1	111	100	92	98	100
K785	123	102	135	115	119
K376	98	93	107		99
K948	113	90	115		106
K806xK584 F_1				109	109
K953				59	59

†Three harvests taken in first year at Waimanalo on 28 Oct. 1993 and 2 Feb. and 5 May 1994.
‡One harvest taken in first year at Mealani on 20 Dec. 1993.
§TANN means for entire data set.
¶Average is the mean of the replicated selections only.
#LSD's for the replicated selections only.

TANN with 118 mg g^{-1}, while K636 had the lowest concentration with 96 mg g^{-1}. At MEA, TANN ranged from 67 to 123 mg g^{-1}, with K156 having the highest concentration (Table 3.8).

A non-significant location and selection by location interaction for TANN allowed the data to be pooled across environments. Based on these findings *L. diversifolia* K749 had the lowest ($P<0.05$) TANN concentrations (Table 3.8).

When analyzed separately by harvest and location, *L. leucocephala* K636 had significantly ($P<0.01$) lower tannin concentrations than all the mean of all other selections once at WAI (Table 3.4). Tannin concentration for K636 was much higher at MEA with 115 g kg^{-1}. Greater differences were evident between K749 and its relatives K156 and K784. At MEA, K156 was highest with TANN concentration of 123 mg g^{-1}, while K749 had the lowest TANN with 67 g kg^{-1}. *Leucaena diversifolia* K749 had lower ($P<0.01$) tannins than either K156 or K784 twice, once at each location, while K156 was different from K784 once (Table 3.4).

Digestibility

Average IVDMD of the forage DM was 100 points higher for all *Leucaena* selections at WAI compared to MEA (689 vs. 591 g kg^{-1}, respectively). The control K636 maintained high digestibility at both locations averaging 734 and 673 g kg^{-1}, respectively (Table 3.9).

Table 3.9. Digestibility of *Leucaena* forage fractions (leaves and small stems) with LSD's from harvest 1 at Waimanalo and harvest 1 at Mealani, Hawaii.

	Location	
	Waimanalo†	Mealani‡
Selection	1	1
	---- g kg^{-1}, DM -----	
Replicated		
K748xK636 F_1	668	647
K748xK584 F_1	620	546
K749	702	488
3way	689	668
KX3 SC	667	554
K806 OP	701	629
K748xK806 F_1	705	592
K748 OP	681	486
K784	598	616
K156	714	632
K806xK636 F_1	776	569
KX2 SC	704	554
K636	734	673
Average§	689	591
$LSD_{0.05}$¶	120	110
Non-replicated		
K804xK636 F_1	756	732
K785	705	568
K376	666	
K948	752	
K806xK584 F_1		626
K953		448

†The first harvest taken at Waimanalo.
‡The first harvest taken at Mealani.
§Averages of replicated selections only.
¶LSD's of replicated selections only.

No differences between selections for IVDMD were detected at WAI (Table 3.4). Digestibility of selection K636 was significantly ($P<0.05$) better than all other selections at MEA (Table 3.4). Digestibility of K749 was lower ($P<0.01$) than both K156 and K784 at MEA, while K748 had lower ($P<0.05$) IVDMD than K748xK806 at MEA (Table 3.4).

Leucaena diversifolia K749, *L. pallida* K748 and K806 x K636 F_1 had the biggest swing in IVDMD between WAI and MEA with a difference of approximately 200 points (702 vs. 488, 681 vs. 486 and 776 vs. 569 g kg^{-1}, respectively) (Table 3.9).

A significant location effect ($P<0.01$) and selection x location interaction ($P<0.05$) for IVDMD required that the data be analyzed separately by location (Table 3.5).

Correlations

Because of the high correlation between forage and total DM, only forage DM correlations will be presented. Forage DM was inversely correlated to psyllid damage ($r= -0.37$, $P<0.05$, $n=42$) once. Forage DM and TANN were correlated once positively and once negatively at WAI ($r=0.36$, $P<0.05$. $n=42$ and $r= -0.44$, $P<0.05$, $n=42$). Psyllid damage was correlated to TANN once ($r=0.32$, $P<0.05$, $n=42$) while TANN were correlated to IVDMD one time at MEA ($r=0.43$, $P<0.01$, $n=43$) (Table 3.10).

Table 3.10. Correlation coefficients for total DM, forage DM, psyllid damage, tannin concentration and IVDMD for *Leucaena* species and hybrids at Waimanalo and Mealani, Hawaii.

Waimanalo†	Harvest				
Correlation	1	2	3	4	5
Total DM					
Forage DM	0.98***	0.97***	0.97***	0.98***	0.98***
Damage	NS	NS	-0.40*	NS	NS
Tannin	NS	0.32*	-0.42*		
IVDMD	NS				
Forage DM					
Damage	NS	NS	-0.37*	NS	NS
Tannin	NS	0.36*	-0.43*		
IVDMD	NS				
Damage					
Tannin	NS	0.32*	NS		
IVDMD	NS				
Tannin					
IVDMD	NS				
Mealani‡					
Total DM					
Forage DM	0.83***	0.92***			
Tannin	NS				
IVDMD	NS				
Forage DM					
Tannin	NS				
IVDMD	NS				
Tannin					
IVDMD	0.43**				

*,**,*** significant at P<0.5, 0.01 and 0.001, respectively, NS = not significant.
†Five harvests taken in first year at Waimanalo on 28 Oct. 1993 and 2 Feb., 5 May, 7 July and 9 Sept. 1994.
‡Two harvests taken in first year at Mealani on 20 Dec. 1993 and 30 June 1994.

DISCUSSION

A significant selection by location interaction is the result of selections like K748xK636 and K748xK584 doing well in both locations while other selections such as K749 and K806xK636 performed well at only one site. The WAI location is situated near sea level and is warm year round, the MEA location is much cooler and has much lower average temperatures. Differences in the number of harvests taken seem to be a direct reflection of temperature and sunlight.

The higher yields of the F_1 hybrids K748xK636 and K806xK636 compared to K636 has been previously reported in Hawaii (Austin, Chapter 2). Yields in this trial were lower than in the earlier trial containing many of the same selections (Austin, Chapter 2). One reason for the difference in yields is that the former experiment received supplemental irrigation for establishment, and because rainfall during establishment and early spring and summer 1994 of this dryland experiment was unexpectedly low (114 mm) from June to September 1993; and 46 mm from March to May 1994). Total DM yields in year 1 at WAI of all *L. pallida* species and hybrids in this experiment was approximately 18 MG ha^{-1} yr^{-1} (8.5 Mg ha^{-1} yr^{-1} forage DM). This is lower than the 40 MG ha^{-1} yr^{-1} (22 Mg ha^{-1} yr^{-1} forage DM) reported from an earlier experiment at the same location. The large difference in biomass yields between the two trials (approximately 20 Mg ha^{-1} yr^{-1}) in the first season suggests

that it makes sense to irrigate when establishing *Leucaena* in Hawaii.

The higher comparative yields of the *L. pallida* x *L. leucocephala* F_1 hybrids at both environments in terms of total DM suggests that future breeding be geared toward developing these hybrids for a wide variety of sites and uses in Hawaii. The reason for top yields at both locations may be due to improved environmental adaptability of the F_1, since the F_1 may contain chromosomes from 4 distinct genomes. *Leucaena pallida* is native to mid-altitude sites in Mexico (approximately 800-1500 m), and is thought to be a naturally occuring hybrid (amphiploid) from parents that are also native to high elevations, *L. diversifolia* ssp. trichandra and *L. esculenta* (Pan and Brewbaker, 1988). *Leucaena leucocephala* is native to lowland sites in Mexico (Brewbaker, 1987b), and is also thought to be a naturally occuring hybrid (amphiploid) from lowland parents as yet undetermined. The higher psyllid resistance of *L. pallida* and better early seedling vigor of *L. pallida* species and hybrids (Sorensson *et al.*, 1994) combined with high productivity of *L. leucocephala* produces a highly adaptable hybrid.

The control K636 produced low quantities of forage DM at both locations. At WAI, psyllid damage may be responsible for the lower yields, although correlations do not necessarily indicate this. The reason why psyllid

damage was not strongly correlated to yield was that all but two of the selections in this trial are considered resistant. K636 had significantly higher damage ratings than all other selections in seven of the 14 months psyllid damage was scored which indicated impeded growth regardless of any correlation. At MEA, the reason for the low total DM yield of K636 (0.2 Mg ha^{-1}) was that it is not tolerant of cooler environments. In subtropical Florida where winter freezing can occur, *L. leucocephala* selections K636, K584 and K8 produced the highest DM yields after a three year study in which psyllid damage was not a significant factor affecting biomass yield (Austin *et al.*, 1995b). However, the maximum summer temperatures in Florida range from 30 to 36 °C, ideal for *L. leucocephala* production. The highest summer temperature at MEA is approximately 24 °C, and the annual average is 16.6 °C. *Leucaena leucocephala* can survive short-duration, below freezing winter temperatures, but produces poorly under a continuously cooler climate. Thus, the cold-tolernat *L. diversifolia* selections in this experiment all produced higher yields at MEA than at WAI. The total DM yield for each harvest of K156, K784 and K749 at WAI was 2.5 Mg ha^{-1} while at MEA it was 3.3 Mg ha^{-1}. The native distribution of *L. diversifolia* species is in cold upland sites (600 to 2300 m.) from Mexico to Honduras (Brewbaker, 1987b).

Psyllid damage ratings were not normally distributed and various transformations could not correct the situation. The reason for the non-normal distribution is that the majority of the *Leucaena* selections in the trial were highly resistant to psyllids, and because a severe epibiotic occurred only once in the 14 month period. In any case, *L. leucocephala* K636 and, to a lesser degree, *L. diversifolia* K156 exhibited higher psyllid damage ratings than the other 11 selections.

K636 had higher psyllid damage ratings than all other selections. This is similar to what Austin *et al.*, (1995b) reported from Florida. *L. diversifolia* K156 was also more susceptible to damage than either K749 or K784. This finding suggests that future breeding of KX3, a cross between tetraploid *L. diversifolia* x *L. leucocephala*, should concentrate on using K784 as one parent. The intermediate level of psyllid damage of both KX2 and KX3 advanced hybrids has also been previously reported (Austin *et al.*, 1995b) from Florida. Both advanced hybrids had higher psyllid damage levels than *L. pallida* species and hybrids, but were not different between themselves. This is in contrast to what Austin *et al.* (1995b) found earlier in Florida, and recently in the African Psyllid Trial (ALPT) conducted at Waimanalo. Both KX2 and KX3 derived from advanced generation composites were selected for high psyllid resistance and vigor. Apparently the effects of the

breeding program concerning these two lines have been successful in reducing the discrepancy between the two hybrids.

Psyllid population dynamics seem to suggest that peak infestation periods occur during the winter months in lowland Hawaii. Higher rainfall and slightly cooler winter temperatures seem to promote psyllid population growth and expansion. Correlations between psyllid damage and biomass yield were only significant at harvest 3, a late winter harvest at WAI. Psyllid damage and yield were negatively correlated in Hawaii both times data were taken on these parameters at WAI in an earlier experiment (Austin, Chapter 2). Significant correlations were also detected all 4 harvests in the ALPT being conducted concurrently with this trial at WAI. The ALPT trial includes several susceptible *Leucaena* selections.

The range in TANN are similar to those reported by Constantinides and Fownes (1994) for *L. leucocephala*, and by Castillo *et al.* (1994) for several lines ranging from 54 to 141 mg g^{-1}. Castillo (1994) reported that *L. leucocephala* selections had the lowest average TANN concentration (66 mg g^{-1}) and that *L. diversifolia* selections had the highest average (120 mg g^{-1}). The relatively low positive correlation coefficient of r=0.32 found only at harvest 2 between psyllid damage and TANN suggests that tannins are not responsible for psyllid resistance, thus negating the

alternative hypothesis that increased tannins are responsible for decreasing psyllid damage. Castillo (1994) also reported a similar correlation coefficient of r=0.33, and suggested that TANN were largely not responsible for psyllid resistance. Interestingly, average TANN concentration for harvest 2 at WAI and TANN concentration at MEA were similar. Harvest 2 coincided approximately with the MEA harvest (20 Dec. vs. 2 Feb) and may indicate an environmental effect on TANN production. Lower winter solar radiation and cooler temperatures at WAI and MEA may lower TANN production.

The significant forage IVDMD location effect and location by selection interaction as evidenced by differences in digestibility can be explained in part by environment. The cooler temperatures may affect the process in which *Leucaena* allocates carbon. Apparently, the cooler climate may trigger the plant to produce greater quantities of cellulose, hemicellulose and lignins, which are inversely correlated to digestibility (Jung, 1989). Generally, cooler temperatures will increase digestibility of grass forages (Akin, 1989; Minson and Wilson, 1980) by reducing the amount of cell wall and its lignification. However, the opposite was observed with *Leucaena* concerning IVDMD at WAI and MEA (689 g kg^{-1} vs. 591 g kg^{-1}, respectively).

IVDMD values for *Leucaena* in this experiment were approximately 10 to 20 percent higher than those reported by

Austin *et al.* (1995a). Some of the same materials tested in Florida were also used in this experiment. In that report the range of IVOMD for eight *Leucaena* spp. and hybrids over three years was 350 to 550 g kg^{-1}. Comparisons between Florida and Hawaii can be made for K636 (521 g kg^{-1} vs. 703 g kg^{-1}, respectively), KX2 (K376 x K8) with 487 vs. 629 g kg^{-1}, KX3 (K156 x K636) with 464 vs. 610 g kg^{-1}, and the single replicated entries *L. pallida* K376 (488 vs. 666 g kg^{-1}) and *L. esculenta* K948 (376 vs. 752 g kg^{-1}).

Differences in digestibility may be due to the type of assay performed, or to differences in the cows rumen microflora. In both cases forage DM was ground to 1 mm diameter and subjected to a 48 hour incubation at 40 °C in rumen fluid. Major differences in technique are associated with the second step of the analysis. In Florida, the sample was subjected to a 44 hour incubation in acid-pepsin (Tilley and Terry, 1963), while in Hawaii the second procedure used a neutral detergent procedure for determining true digestibility (Goering and Van Soest, 1970). Digestibility was not correlated to any other parameters at WAI however, a positive correlation was detected at MEA with TANN.

CONCLUSION

The F_1 hybrids K748xK636 and K748xK584 outperformed all other selections at both locations in the first season and beyond. The importance of this finding is that the breeding

program can develop one or two forage varieties for several environments in Hawaii. The genetic makeup of the F_1 hybrids are based on 4 separate genomes that confer greater environmental adaptibility. The good psyllid resistance and vigor of *L. pallida* when crossed to the high productivity (in the absence of psyllids) and good digestibility of *L. leucocephala* make these hybrids ideal. Psyllid resistance should be developed for lowland, tropical environments while cool tolerance is required for higher altitudes. Better performance of *L. diversifolia* K749 and K784 in terms of psyllid resistance at WAI and at higher elevations suggests the use of these lines for hybridization with *L. leucocephala* to produce a new family of KX3 hybrids. More research on the relationship of TANN to digestibility and psyllid damage is required. The high digestibility of all *Leucaena* selections especially the F_1 hybrids is good news for forage production schemes.

CHAPTER 4

THE AFRICA *LEUCAENA* PSYLLID TRIALS (ALPT)

ABSTRACT

The genus *Leucaena* includes 15 to 16 recognized species that differ in resistance to the leucaena psyllid (*Heteropsylla cubana* Crawford). These trials were conducted to identify sources of psyllid resistance and determine total biomass yield of various *Leucaena* species and hybrids in Kenya and Malawi, Africa. We also wanted to test the durability of psyllid resistance between areas of new and old infestations. Twenty different *Leucaena* spp. and hybrids were investigated for 16 months at the Waimanalo Research Station, Hawaii. Tested were 5 *L. leucocephala*, 5 *L. pallida*, 1 *L. esculenta*, and 2 *L. diversifolia* species. In addition, there were advanced progeny hybrids such as *L. pallida* x *L. diversifolia* KX1, *L. pallida* x *L. leucocephala* KX2, a KX2 special composite from superior F_4 trees, KX2 progenies 6-15 and 5-36, *L. diversifolia* x *L. leucocephala* KX3 and KX3 progeny 91-13. *Leucaena esculenta* and *L. pallida* consistently displayed the lowest psyllid damage. *Leucaena leucocephala* selections had the highest damage ratings over the 14 month period, while the F_2 hybrids had intermediate levels of damage. Psyllid damage was negetively correlated ($P<0.01$) with total biomass four of

the five harvests. Psyllid damage ratings at Machakos, Kenya were similar to Waimanalo observations. The highest damage ratings were for *L. leucocephala* genotypes, while *L. esculenta* and the *L. pallida* selections were lowest. Total wet biomass yields ranged from 10.8 to 78.9 Mg ha^{-1} over five harvests (3.2 to 23.7 Mg ha^{-1} total DM). The best yielding selections were the *L. pallida* entries. As a group *L. pallida* K376, K748, K824 and K953 yielded 72.2 Mg ha^{-1} (21.6 Mg ha^{-1} DM), as compared to the giant *L. leucocephala* selections K565, K584, K636 and K584xK636 which yielded 43.9 Mg ha^{-1} total wet biomass (13.2 Mg ha^{-1} DM). *Leucaena diversifolia* K156 and K784 yielded 34 Mg ha^{-1} (10.2 Mg ha^{-1} DM), while the advanced progeny hybrids KX1, KX2 and KX3 as a group yielded 51.5 Mg ha^{-1} total wet biomass (15.4 Mg ha^{-1} DM). At Machakos, Kenya, total DM biomass did not differ from harvest 1 and ranged from 2.4 to 6.2 Mg ha^{-1}. *Leucaena pallida* selections should be considered as future parent material for developing crosses between *L. pallida* x *L. leucocephala* for psyllid infested environments.

INTRODUCTION

Many species of *Leucaena* are susceptible to psyllids, an insect pest that has recently invaded Africa (Van Den Beldt and Napompeth, 1992). The most commonly grown species of leucaena in the world, *L. leucocephala* (Lam.) de Wit, is highly susceptible to psyllid damage (Brewbaker *et al.*,

1989). When newly introduced, psyllids cause extensive damage to susceptible varieties in areas devoid of biological controls (Nakahara and Funasaki, 1986). However, infestation peaks begin to level off after the first or second full season (Van Den Beldt and Napompeth, 1992). This is especially true when *Leucaena* spp. are managed for wood instead of forage (Van den Beldt and Napompeth, 1992). An example is that of Florida, where early psyllid infestation caused tremendous damage after being first reported in 1983 (Othman and Prine, 1984). Psyllids caused the complete defoliation of several accessions at that time. The arrival of psyllids to Hawaii in 1984 caused extensive damage to susceptible *Leucaena* stands (Nakahara and Lai, 1984). Severe psyllid infestations have also been reported in the Pacific Basin, Southeast Asia and Australia. Psyllids were first reported in the Mascarene islands in late 1991 (Vandeschricke *et al.*, 1992) and invaded Africa in 1992 (Van den Beldt and Nampopeth, 1992).

Psyllids are native to Mexico and Central America and are members of the order Homoptera and family Psyllidae (Beardsley, 1986). Psyllids infest the young, actively growing tips of the plant, feeding and ovipositing in these areas (Beardsley, 1986). Psyllid populations fluctuate tremendously and seem to depend on a complex set of interactions between plant growth, climatic factors, and biological controls (Bray and Woodroffe, 1988; NFTA, 1988).

There are conflicting reports as to the effect of moisture on psyllid populations. Shivamurthy *et al.* (1991) observed increased psyllid populations during and just after wetter periods in India, while Vivekanandan and Bandara (1990) in Sri Lanka reported a decrease in the psyllid population and corresponding damage levels during the wet season. An abundance of young leaf material associated with forage managed *L. leucocephala* provides optimal conditions to support high psyllid populations. Psyllid populations fluctuate tremendously in Hawaii with peak infestations occurring in the rainy seasons between November to March (Wheeler, 1998; M.T. Austin, Chapter 3).

Tree and shrub forage legumes are increasingly being emphasized for ruminant production in tropical and subtropical regions (Shelton *et al.*, 1992). In many instances, legume trees are the only source of protein in tropical grasslands because of the difficulties in establishing and maintaining herbaceous legumes. *Leucaena leucocephala* is highly regarded as a ruminant livestock forage and is commonly grown around the world (Brewbaker *et al.*, 1989; Shelton and Brewbaker, 1994). Psyllid damage was inversely correlated to biomass yield in a trial conducted in 1991 at Waimanalo (Austin, Chapter 2). Varieties with the highest resistance and yield were all *L. pallida* species and hybrids (Austin, Chapter 2). In Hawaii up to 30 Mg ha^{-1} yr^{-1} forage DM was reported for the best yielding *L.*

leucocephala selections prior to the invasion of the psyllid in 1984 (Brewbaker *et al.*, 1972). Total DM biomass yields of 20.8 Mg ha^{-1} yr^{-1} (12.3 Mg ha^{-1} yr^{-1} forage DM) of *L. leucocephala* K8 at 45,000 plants ha^{-1} under psyllid-free conditions and growing in an arid environment have been reported for the island of Hawaii (Guevarra *et al.*, 1987). Jama and Nair (1989) report DM forage yield of 1.4 Mg ha^{-1} yr^{-1} of *L. leucocephala* at Machakos, Kenya, over 5 harvests yr^{-1}, which are extremely low yields in comparison to other studies.

One approach for reducing psyllid damage is to identify resistance in the genus and develop resistant forage lines either through hybridization or species level improvement. Variability for psyllid resistance in the genus is extensive (Brewbaker *et al.*, 1989). Resistant species include *L. pallida*, *L. esculenta* and *L. collinsii* (Sorensson and Brewbaker, 1986), but it is the tetraploid *L. pallida* that is best suited for developing fertile hybrids with *L. leucocephala*. *Leucaena pallida* is thought to be an amphidiploid between *L. esculenta* n=26 x *L. diversifolia* ssp. *trichandra* n=26 (Pan, 1985; Pan and Brewbaker, 1988). Both *L. pallida* and *L. esculenta* exhibited excellent psyllid resistance over several environments (Austin *et al.*, 1995a). Reports stemming from the International *Leucaena* Psyllid Trials indicate that resistant lines maintain their resistance in different environments (NFTA, 1988).

Biological controls of psyllids have been identified and consist of a variety of predacious and parasitic enemies (Nakahara and Funasaki, 1986) that may reduce psyllid pressure on the plant. Populations of biological control agents such as ladybird beetles (Coleoptera: Coccinellidae) generally lag behind psyllid populations due to the rapid life cycle of the psyllid (10.4 d to adult; Takahara *et al.*, 1990) compared to ladybird beetles (30.5 d to adult, Nagamine, 1986). This delayed response accounts for the reduced ability of the predator to prevent population explosions and consequent plant damage (Funasaki *et al.*, 1989).

The purpose of this study was to identify *Leucaena* spp. and hybrids for psyllid resistance and high biomass productivity in diverse tropical environments with the intention of identifying the best yielding selections under psyllid pressure in order to develop suitable breeding objectives for African conditions.

MATERIALS AND METHODS

Waimanalo

The Waimanalo Research Station on Oahu is located at 21° 20′ N, 158° 20′ W with a mean elevation of 20 m above sea level, average rainfall of 1380 mm yr^{-1} and temperatures ranging from 22-27 °C. Soils are fertile and consist of

basaltic colluvium underlaid by coral with a soil pH of approximately 6.0 to 6.5.

Seeds from 20 *Leucaena* selections (Table 4.1) were scarified in boiling water for 5-10 seconds, rinsed, and soaked in tap water overnight. Seeds were inoculated with *Leucaena* rhizobium (TAL 1145, NiFTAL Project, Univ. of Hawaii) and planted March 1993 into dibble tubes containing commercial planting medium (75% peat, 25% perlite). Seedlings were watered daily in the greenhouse until ready for transplanting. Seedlings were transplanted 16 June 1993 into an Isohyperthermic Vertic Haplustoll. No irrigation was used.

The experimental design was an augmented randomized complete block. Fifteen selection were replicated 3 times and the remaining five selections replicated once. Seedlings were planted in double rows 1 meter apart and 0.25 m within the row. Plots were separated by 1.5 m producing a plant population of 35,500 plants ha^{-1}.

Plants were harvested three times for biomass determination the first year on 1 Nov. 1993 and 3 March, and 6 June 1994. The second year harvests were taken on 9 August and 1 Oct. 1994. Plant heights averaged 2 m at harvest. A total of eight plants were harvested four from each side of the double row, along a 1 m linear distance within the row and cut at 0.5 m above ground level. Material was weighed wet in the field.

Table 4.1. *Leucaena* selections growing at Waimanalo Research Station, Hawaii.

Replicated Entries:

KX1 *L. pallida* x *L. diversifolia* K376 x K156 (F_3)†
KX2 *L. pallida* x *l. leucocephala* K376 x K8 (F_4)
KX3 *L. leucocephala* x *L. diversifolia* K636 x K156 (F_3)
K156 *L. diversifolia* (4n)
K784 *L. diversifolia* (4n)
K636 *L. leucocephala*
K584 *L. leucocephala*‡
K565 *L. leucocephala*
K584 x K636 *L. leucocephala* (F_2)
K376 *L. pallida*
K824 *L. pallida*
K953 *L. pallida*
K748 *L. pallida*‡
K948 *L. esculenta* (psyllid resistant control)
K997 *L. leucocephala* (psyllid susceptible control)

Non-replicated

KX2 Tree 6-15 *L. pallida* x *l. leucocephala* K376 x K8 (F_4)
KX2 Tree 5-36 *L. pallida* x *l. leucocephala* K376 x K8 (F_4)
KX2 sc *L. pallida* x *l. leucocephala* K376 x K8 (F_4)
KX3 Tree 91-13 *L. leucocephala* x *L. diversifolia* K636 x K156 (F_3)
K820 *L. pallida*

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†K numbers represent University of Hawaii nomenclature.
‡These selections (K584 and K748) were replicated twice only.

Total wet biomass was analyzed as a strip-block with harvest as the main plot, selection as the sub-plot, and selection by harvest as the sub-sub plot. If a selection by harvest interaction was significant or if harvests were different, data were then analyzed separately by harvest and analyzed as a RCB. LSD was used to separate means for each harvest and single degree orthogonal contrasts were used for mean separations of grouped total biomass data. Total wet biomass was multiplied by 0.3 in order to present approximate total DM yields.

Psyllid damage ratings were taken monthly. Leaf tips of 6 plants per plot (18 plants/3 $reps^{-1}$) were examined for plant damage using an empirical scale of 1 to 9 as described by Glover (1988), where 1= no damage, and 9= complete defoliation. A square root transformation was used on psyllid damage to correct for a non-normally distributed rating scale (Steele and Torrie, 1980). If the variances were still non-normally distributed after transformation then data were analyzed using Proc NPAR1WAY (SAS, 1986), using Wilcoxon scores, a method of ranking the data that doesn't require a normal distribution to determine treatment differences. Mean separations were conducted using Kruskal-Wallis chi square on the ranked data.

Pearson correlation coefficients (SAS, 1986) were determined at each harvest between psyllid damage and total biomass. The highest psyllid rating for each harvest

interval was used for correlating total biomass with damage.

The African Sites

Because of difficulty in establishing these trials at three of the four sites, only Machakos, Kenya has reported results at the time of this dissertation.

Machakos, Kenya

Nineteen *Leucaena* selections (Appendix C) were planted at Machakos, Kenya in November 1993 into a RCB with three replications. The first harvest was taken on 26 July 1994 when plant height was approximately 2 to 2.5 m. Plants were scored using the same psyllid rating scale that was used at Waimanalo.

Total biomass was analyzed as a RCB using Proc GLM (SAS, 1986). Mean separations were performed using LSD.

Psyllid data were analyzed separately at each rating period. If a non-normal distribution of the variances was detected then data were analyzed using PROC NPAR1WAY (SAS, 1986), a method of rank analysis.

RESULTS

Only one biomass harvest and three monthly psyllid ratings from Machakos, Kenya had been received as of November 1994. These data have been incorporated into the

paper, but we are awaiting further information before analyzing further.

Biomass yields

Total DM biomass yields at Machakos, Kenya in harvest 1 and ranged from 2.4 to 6.2 Mg ha^{-1} for K948 and KX1, respectively (Table 4.2), treatments did not differ in harvest one (Table 4.3).

The total wet biomass yields over the first five harvests at Waimanalo ranged from 10.8 to 78.9 Mg ha^{-1} (3.2 to 23.7 Mg ha^{-1} DM) (Table 4.4). The highest yielding selections were all *L. pallida* species. When averaged they produced 72.2 Mg ha^{-1} (21.6 Mg ha^{-1} DM) (Table 4.4).

Leucaena pallida K953 was the lowest yielding selection of the *L. pallida*, producing lower biomass yields ($P<0.001$) once at harvest 2 when compared against the mean value of the remaining *L. pallida* selections (Table 4.5). The other *L. pallida* selections were not different from each other over the five harvests. The next highest yielding entries were the advanced *Leucaena* hybrids KX1, KX2 and KX3 averaging 51.5 Mg ha^{-1} (15.4 Mg ha^{-1} DM) (Table 4.5). Total biomass yield of the advanced hybrids differed once at harvest 5 (Table 4.5). Both *L. leucocephala* and *L. diversifolia* groups produced the lowest yields at 37.3 and 34.0 Mg ha^{-1} (11.2 and 10.2 Mg ha^{-1} DM), respectively (Table 4.5).

Table 4.2. Total DM biomass yield Mg ha^{-1} for harvest 1† at Machakos, Kenya

Selection		Total DM	
Replicated			
KX1‡	PALLxDIV4	6.2	NS
K565‡	LEUC	5.9	
K748‡	PALL	4.9	
K953‡	PALL	4.6	
K824	PALL	4.4	
K784	DIV4	4.3	
K376‡	PALL	3.9	
K584	LEUC	3.7	
K156	DIV4	3.3	
KX3‡	DIV4xLEUC	3.1	
K584xK636	LEUCxLEUC	3.0	
K948	ESCU	2.4	
Non-replicated			
K806	PALL	3.6	
K636	LEUC	2.7	
KX2	PALLxLEUC	1.3	
KX2sc	PALLxLEUC	1.0	
K997	LEUC	0.5	

NS = not significant.
†Harvest was taken 26 July 1994, approximately 8 months after planting.
‡Replicated twice only, all others replicated thrice.

Table 4.3. Anova of total biomass yields at Machakos, Kenya harvest 1 using type III sum of squares.

Source	df	MS (10^{-6})	$F_{0.05}$
BLK	2	11.92	6.70 **
SEL.	11	2.45	1.38 NS
ERROR	16	1.78	
TOTAL	29		

** Significant at 0.01 probability level, NS not significant.

Table 4.4. Total biomass yields Mg ha^{-1} with LSD's for five harvests at Waimanalo, Hawaii.

Selection		---Biomass Harvests†--- 1	2	3	4	5	Tot. Biom.	Tot‡ DM
Replicated								
K824	PALL	10.9	23.1	23.1	11.9	6.8	75.8	22.7
K748	PALL§	13.3	20.4	24.4	12.0	8.2	78.3	23.5
K376	PALL	11.9	20.3	25.5	11.9	9.3	78.9	23.7
K953	PALL	14.1	10.7	16.7	11.1	9.7	62.3	18.7
K948	ESCU	10.1	11.1	14.1	6.9	5.6	47.8	14.3
KX3	DIV4xLEUC	9.5	14.9	6.7	8.8	8.3	48.2	14.5
KX2	PALLxLEUC	8.1	26.3	10.7	8.9	7.7	61.7	18.5
KX1	PALLxDIV4	7.7	13.2	12.0	6.9	4.7	44.5	13.4
K636	LEUC	7.5	18.3	5.7	6.7	9.1	47.3	14.2
K584	LEUC§	5.4	8.4	5.8	5.3	7.5	32.4	9.7
K565	LEUC	7.3	20.0	6.7	9.0	9.3	52.3	15.7
K584xK636	LEUC	5.7	16.1	5.7	8.1	8.1	43.7	13.1
K997	LEUC	1.8	4.3	1.7	1.1	1.9	10.8	3.2
K784	DIV4	5.9	14.6	11.4	4.6	3.3	39.8	11.9
K156	DIV4	5.3	7.9	5.9	4.2	4.9	28.2	8.5
Average¶		8.3	15.5	11.7	7.8	6.9	49.7	
$LSD_{0.05}$#		7.0	7.1	7.9	4.5	3.6	5.5	
Non-replicated								
KX3 91-13	DIV4xLEUC	12.8	40.8	22.0	17.8	11.2	104.6	31.4
KX2 sc	PALLxLEUC	14.4	36.8	18.8	14.0	7.6	91.6	27.5
K820	PALL	10.8	28.2	26.8	6.2	6.6	78.6	23.6
KX2 6-15	PALLxLEUC	7.8	32.0	7.2	6.4	4.6	58.0	17.4
KX2 5-36	PALLxLEUC	10.0	22.8	9.2	9.0	8.2	59.2	17.8

†Harvests occurred on 1 Nov. 1993, and 3 Mar., 6 June, 9 Aug. and 18 Oct. 1994.
‡Total DM is the wet total biomass multiplied by 0.3.
§Replicated twice only.
¶Average of replicated selections only.
#LSD's of replicated selections only.

Table 4.5. Contrasts of total wet biomass yields from 1993 to 1994 at Waimanalo, Hawaii.

Contrast	Harvest† 1	2	3	4	5
K948 vs. All	NS	NS	NS	NS	NS
LEUCS vs All-K948	**	*	***	**	NS
K997 vs. LEUCS	NS	***	***	**	***
LHYB vs. K636, K584 and K565	NS	NS	NS	NS	NS
K565 vs. K636, K584	NS	NS	NS	NS	NS
K636 vs. K584	NS	*	NS	NS	NS
DIV4 vs. PALLS‡	**	**	***	***	***
K784 vs. K156	NS	NS	NS	NS	NS
PALLS vs. ADVHYBS§	*	NS	***	**	NS
KX3 vs. KX1, KX2	NS	NS	NS	NS	*
KX1 vs. KX2	NS	NS	NS	NS	*
K953 vs. PALLS-K953	NS	***	NS	NS	NS
K376 vs. K824, K748	NS	NS	NS	NS	NS
K824 vs. K748	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, NS = not significant.
†Harvests correspond to Nov 1993, Mar, June, Aug and Oct 1994.
‡PALLS are K953, K376, K824, K748.
§ADVHYB is the advanced hybrid selections KX1, KX2 and KX3.

Leucaena leucocephala K997 "common" had lower yields ($P<0.05$) than the mean of the other *L. leucocephala* entries four of the five harvests (Table 4.5). If K997 is omitted, since it is regarded as an unimproved selection, then the remaining *L. leucocephala* selections produced 43.9 Mg ha^{-1} (13.2 Mg ha^{-1} DM). The remaining *L. leucocephala* selections did not differ with respect to yield with the exception of harvest 2 where K636 had a higher yield than K584 (Table 4.5). *Leucaena diversifolia* K156 and K784 did not differ in yield. The remaining species *L. esculenta* K948 produced 47.8 Mg ha^{-1} (14.3 Mg ha^{-1} DM) and was not different from the other selections at all harvests (Table 4.5). The non-replicated selections produced high total wet biomass yields. The best, KX3 91-13 produced 104.6 Mg ha^{-1} (31.4 Mg ha^{-1} DM) while KX2 6-15 produced the lowest total biomass with 58 Mg ha^{-1} (17.4 Mg ha^{-1} DM) (Table 4.4).

Harvest and harvest by selection were significant ($P<0.001$) when combined over the five harvests at Waimanalo (Table 4.6). As a result, harvests were analyzed separately (Table 4.7).

Psyllid resistance

Psyllid damage fluctuated tremendously throughout the first year at Waimanalo (Figure 4.1). Of 14 monthly observations, five months had damage ratings of 3 or less

Table 4.6. ANOVA of combined† total wet biomass harvests and psyllid damage of ALPT at Waimanalo, Hawaii, using type III sum of squares.

	df	Total Biomass MS (10^{-6})		Psyllid Damage MS Value	
BLK	2	20.38	NS	0.49	*
HARVEST	4	536.75	***	158.54	***
BLK*HARV.	8	25.27		0.27	
SELECTION	14	212.23	***	116.68	***
BLK*SEL‡	26	33.41		0.27	
SEL*HARV	56	36.98	***	72.51	***
ERROR c	104	8.18		1.10	
TOTAL	214				

*, ***, significant at P< 0.05 and 0.001 probability level.
†Includes harvests 1-5 of replicated selections only, no augments.
‡Two selections (K584 and K748) were replicated only twice.

Table 4.7. ANOVA of individual total wet biomass† by harvest of ALPT at Waimanalo, Hawaii using type III sum of squares.

		MS Values (10^{-6})				
		---------- Harvest ----------				
Source	df	1	2	3	4	5
BLK	2	74.67**	32.54 NS	5.10 NS	1.36 NS	7.80 NS
SEL.	14	29.12*	114.84***	170.00***	29.15**	17.03**
ERROR‡	26	13.14	16.84	23.66	7.94	4.54
TOTAL	42					

*, **, *** significant at 0.05, 0.01 and 0.001 probability level, NS= not significant.
†Only replicated selections were analyzed, no augments.
‡Two selections (K584 and K748) were replicated only twice.

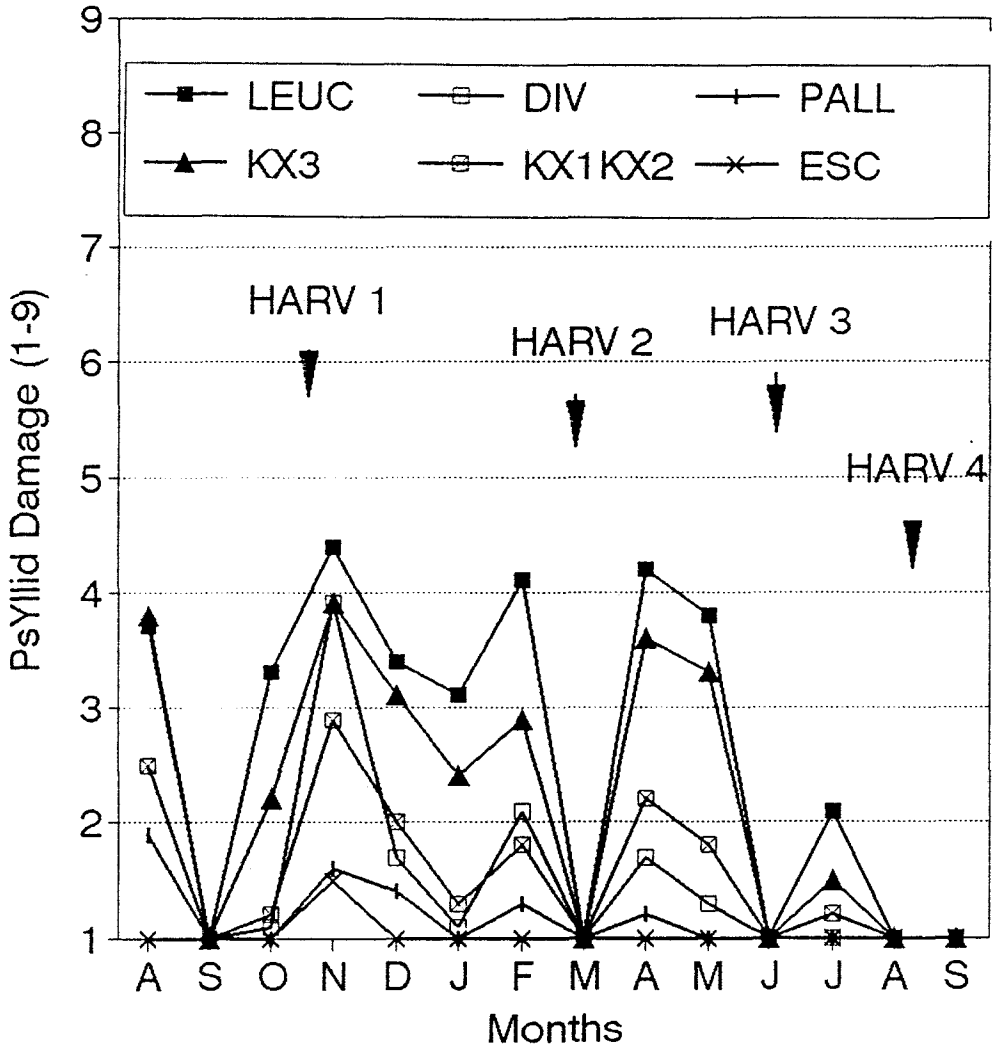

Figure 4.1. *Leucaena* psyllid damage from August 1993 to Sept. 1994 in Waimanalo. LEUC=*L. leucocephala* selections K565, K584, K636, K997 and K584xK636 F_2; DIV=*L. diversifolia* K156 and K784; PALL=*L. pallida* K376, K748, K824 and K953, ESC=*L. esculenta* K948; KX3=*L.diversifolia* x *L. leucocephala* F_3 (K156xK636); KX2=*L. pallida* x *L. leucocephala* F_4 (K376xK8); KX1=*L. pallida* x *L. diversifolia* (K376xK156).

Table 4.8. Non-parametric analysis (Kruskal-Wallis chi-square test) on psyllid damage ratings derived from Wilcoxon Rankings at Waimanalo over 14 months from 1993 to 1994.

	Month†							
Chi-Square Test‡	1	3	4	5	6	7	9	10
K565 vs K636	***	***	**	***	***	NS	NS	NS
K784 vs K156	***	NS	*	**	NS	***	**	**
KX3 vs KX2, KX1	**	***	*	***	***	***	***	***
KX1 vs KX2	NS	*	NS	NS	NS	*	NS	NS
K953 vs K376, K824 and K748	***	NS	***	***	NS	***	**	NS
K376 vs K824, K748	NS	NS	***	NS	NS	NS	NS	NS

*, **, *** Significant at the P< 0.05, 0.01 and 0.001 probability level, NS = not significant.
†Months correspond to Aug, Oct, Nov, Dec, 1993 and Jan, Feb, Apr. May, 1994 When psyllid damage ratings were 3 or higher.
‡Chi-square tests were based on mean rankings of the various comparisons were developed using Wilcoxon Scores in Proc NPAR1WAY in SAS. Significant values indicate that these particular comparisons differed in psyllid damage.

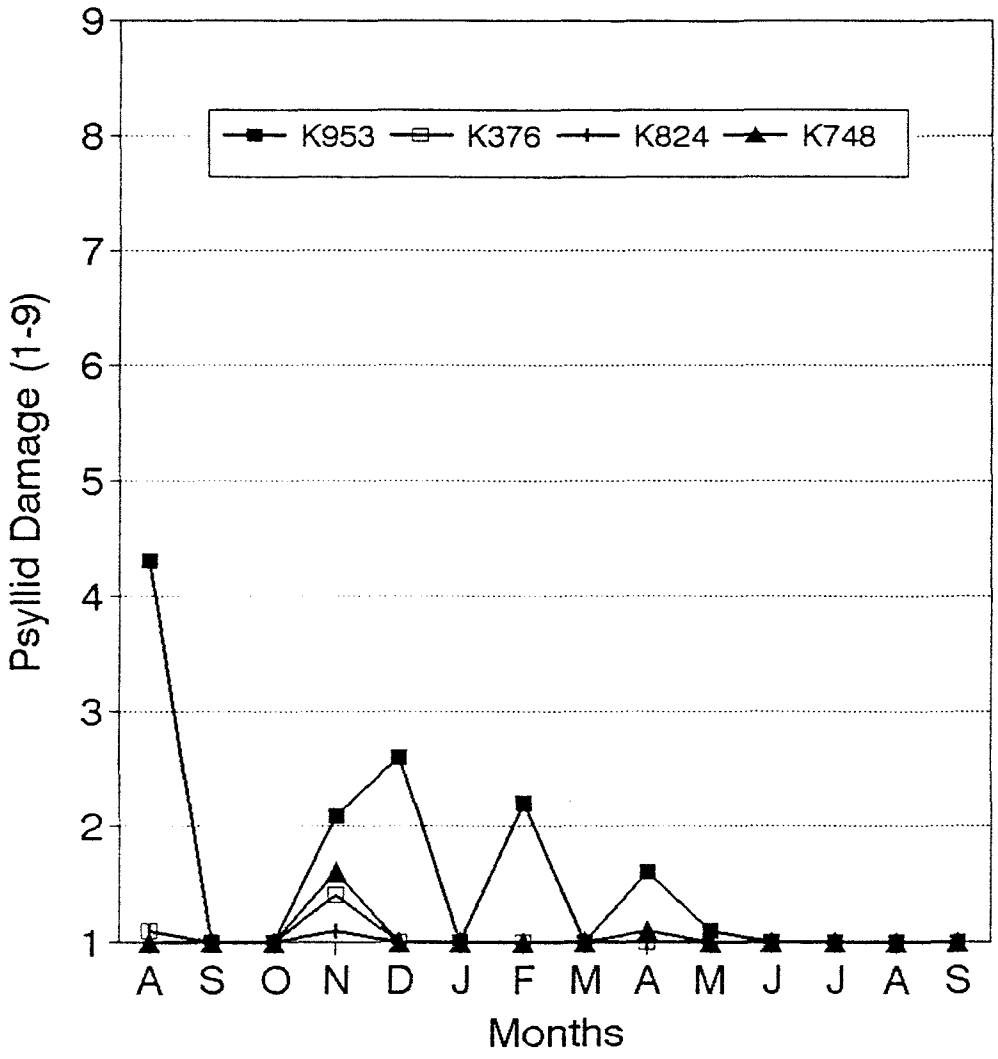

Figure 4.2. *Leucaena pallida* psyllid damage at Waimanalo from August 1993 to September 1994. *L. pallida* K376, K748, K824 and K953.

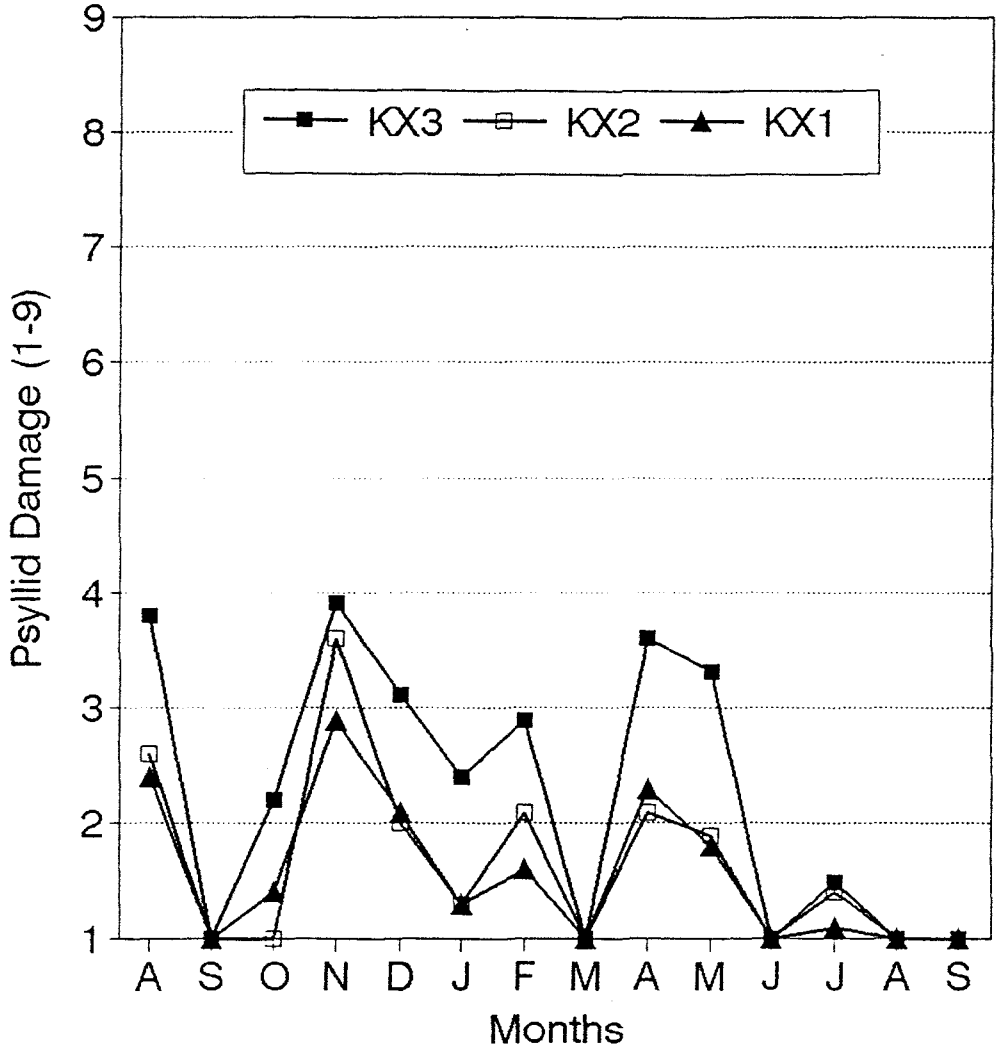

Figure 4.3. Advanced progeny *Leucaena* psyllid damage at Waimanalo from August 1993 to Sept. 1994. KX3 = *L.diversifolia* x *l. leucocephala* F_3 (K156xK636); KX2 = *L. pallida* x *L. leucocephala* F_4 (K376xK8); KX1 = *L. pallida* x *L. diversifolia* (K376xK156).

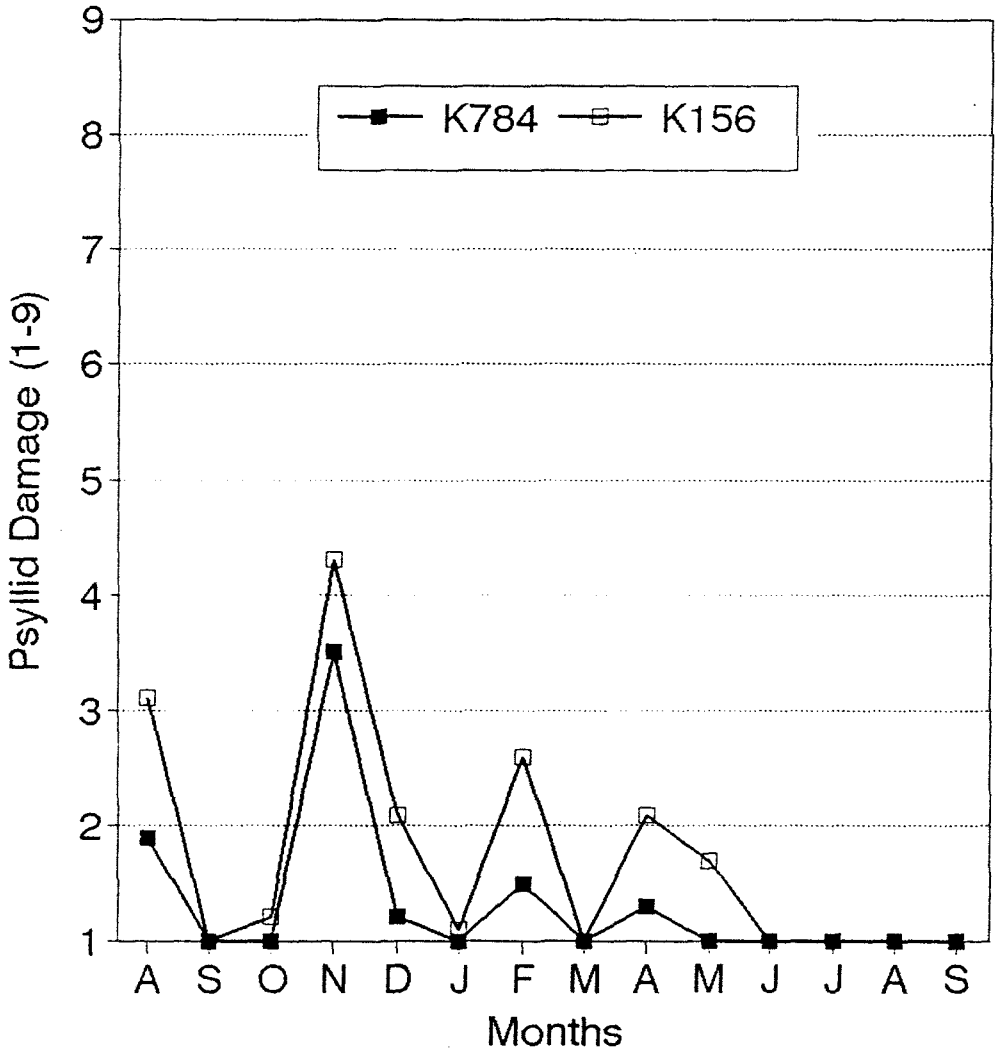

Figure 4.4. *Leucaena diversifolia* psyllid damage at Waimanalo from August 1993 to Sept. 1994. *L. diversifolia* K156 and K784.

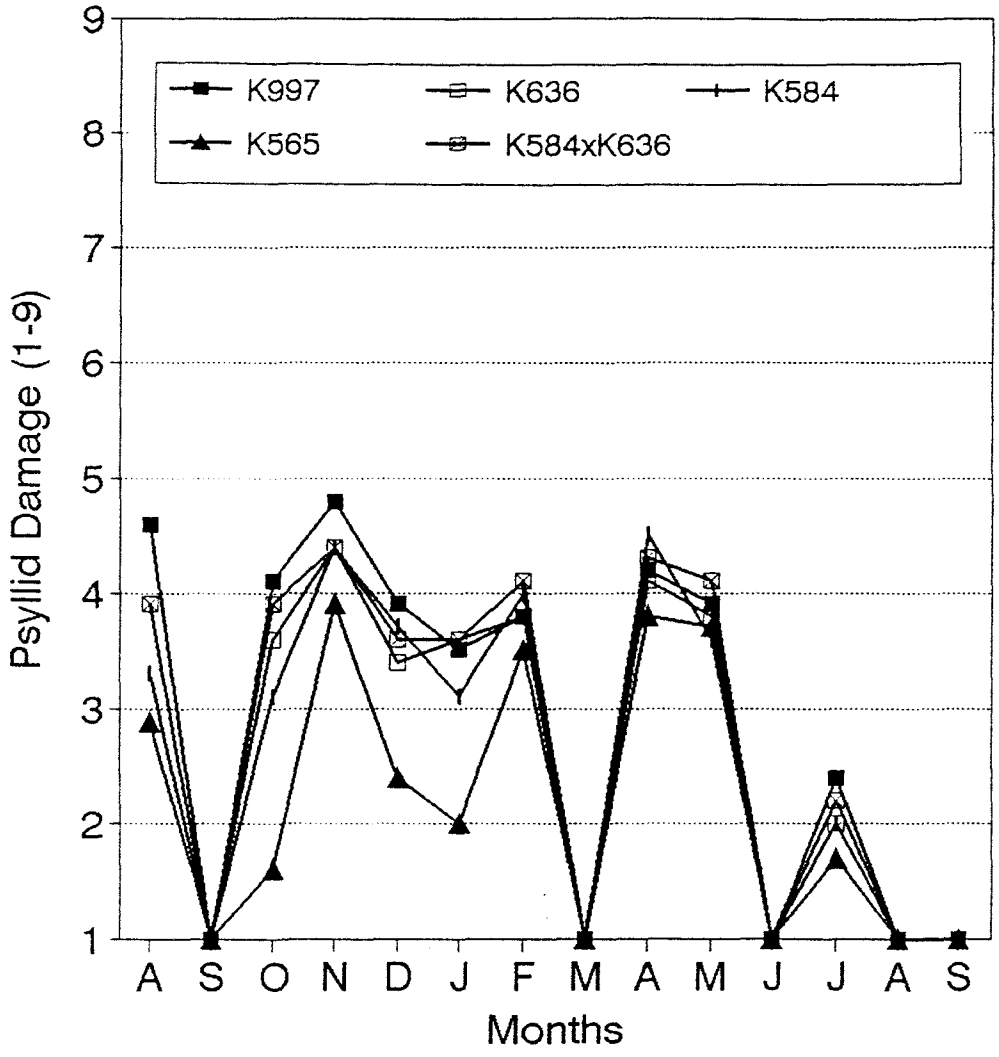

Figure 4.5. *Leucaena leucocephala* psyllid damage at Waimanalo from August 1993 to Sept. 1994. *L. leucocephala* selections K565, K584, K636, K997 and K584xK636 F_2 .

(Figure 4.1). As a result, only months where psyllid damage was three or higher were further analyzed (Table 4.8). Low psyllid damage ratings were the result of either harvesting or hot, dry summer conditions.

Leucaena esculenta K948 had the best overall psyllid resistance with virtually zero damage throughout the trial (Figure 4.1). The *L. pallida* selections also had low damage ratings with the exception of K953 (Figures 4.1 and 4.2). *L. pallida* K953 had higher damage ratings ($P<0.01$) than K748, K376 and K824 combined in five of the eight high psyllid damage periods (Table 4.8). The advanced hybrids KX1, KX2 and KX3 had intermediate damage levels (Figure 4.1). Advanced hybrid KX3 had higher damage ratings ($P<0.05$) than either KX1 and KX2 eight times, while KX1 and KX2 differed ($P<0.05$) twice (Table 4.8, Figure 4.3). *Leucaena diversifolia* selection K784 had lower damage ratings six times compared to K156 (Table 4.8, Figure 4.4).

The *L. leucocephala* selections all had high damage ratings with the exceptions, of K565 which differed from K636 five times (Table 4.8, Figure 4.5).

At Machakos psyllid damage was high and treatment differences were detected ($P<0.001$) for each of the three observational dates. As found elsewhere, the *L. leucocephala* genotypes had the highest psyllid damage (Figure 4.6) while *L. esculenta* and *L. pallida* species were lowest. The advanced hybrids had intermediate damage

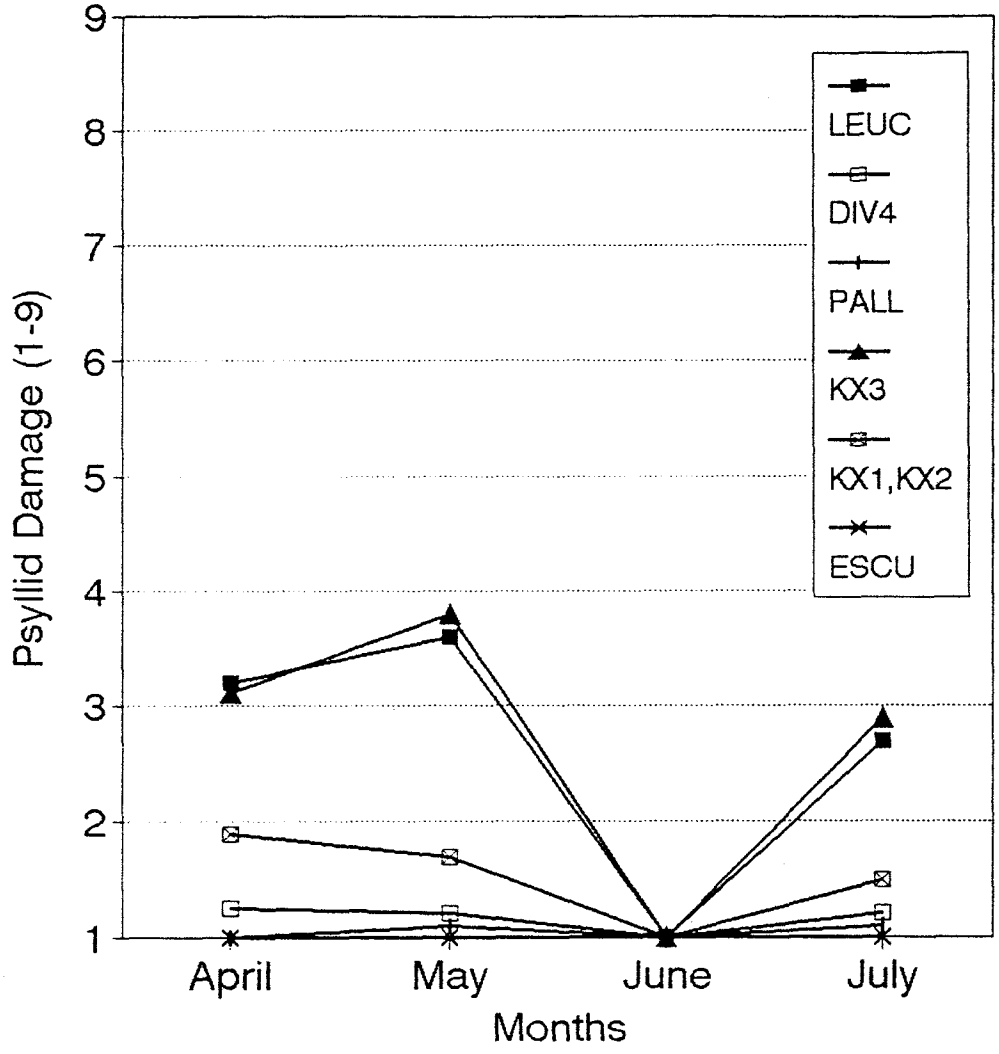

Figure 4.6. *Leucaena* psyllid damage at Machakos, Kenya for three months in 1994. LEUC= *L. leucocephala* K565, K584, K636, K997 and K584xK636; DIV4= *L. diversifolia* K156 and K784; PALL= *L. pallida* K376, K748, K806, K824 and K953; ESC= *L. esculenta* K948; KX1 and KX2= *L.pallida* x *L. diversifolia* (K376 x K156 F_3) and *L. pallida* x *L. leucocephala* (K376 x K8 F_3), respectively; KX3= *L. diversifolia* x *L. leucocephala* (K156 x K636 F_3).

Table 4.9. Correlations between total biomass and psyllid damage average between harvest intervals at Waimanalo, Hawaii. From replicated selections only (N=43).

	Harvest				
Correlation	1	2	3	4	5
Total Biomass					
Psyllid Damage	-0.44**	-0.38*	-0.72***	-0.38*	NS

, * Significant at the 0.05, 0.01 and 0.001 probability level, NS=not significant.

levels, while the *L. diversifolia* genotypes had minimal damage.

Psyllid damage ratings did not normalize at Waimanalo for all 14 monthly observations nor were ratings corrected by a square root transformation. Leucaena selections were highly significant ($P<0.0001$) for damage in nine of the 14 months when analyzed non-parametrically. The exception were months when psyllid damage ratings were 1 over the entire trial.

Correlations

Psyllid damage average between harvest intervals and total biomass yield were negatively correlated four of the five harvests (Table 4.9). They were not correlated at harvest five because psyllid damage levels for the entire trial were = 1, i.e. no detectable damage (Figure 4.1).

DISCUSSION

Total biomass yields were lower than expected due to drought. Rainfall was minimal during the summer establishment period (116 mm), and again during the spring and summer of 1994 (68 mm). Because of low rainfall, harvest number in year 1 was reduced to three as compared to 5 over a 13 month period as previously reported by Austin (Chapter 2) at Waimanalo, although plant establishment was high. The lower yields of this study suggest that

irrigation may be necessary to produce top yields. At least irrigation at time of establishment may be economically attractive.

The high yields of *L. pallida* were related to its better psyllid resistance. The inverse correlation of yield and resistance detected in four of the five harvests signifies the importance of psyllid resistance when growing *Leucaena* in lowland tropical environments. Higher yields, good psyllid resistance and inverse correlations between yield and psyllid damage were reported earlier at Waimanalo by Austin (Chapter 2) for *L. pallida* as a group. In that study, *L. pallida* species and F_1 hybrids, with *L. pallida* produced 40 Mg ha^{-1} total DM (37 Mg ha^{-1} yr^{-1}). *Leucaena pallida* yields from this study were 72.2 Mg ha^{-1} total wet biomass (21.6 Mg ha^{-1} DM) over an 18 month period. The total DM biomass yield of *L. leucocephala* K636 in the earlier experiment was 12.7 Mg ha^{-1} (11.7 Mg ha^{-1} yr^{-1}) which was also higher than the present study at 8.6 Mg ha^{-1} yr^{-1} DM.

The significantly higher yields of *L. pallida* even under severe environmental stress as compared to the *L. leucocephala* selections represents their importance in the overall breeding scheme at the University of Hawaii, and for developing improved *Leucaena* varieties for Africa. F_1 hybrids between *L. pallida* and *L. leucocephala* have produced the highest yields in Hawaii. In a separate, concurrent forage trial at Waimanalo, yields of the interspecific F_1

hybrid K748xK636 were 26 Mg ha^{-1} yr^{-1} total DM. Interestingly, this hybrid and K748xK584 were also the top yielding entries in a distinctly different environment (850 m) at Mealani, Hawaii (Austin, Chapter 3). K748xK636 also produced the highest yields at Waimanalo from 1991 to 1992, producing 59 Mg ha^{-1} yr^{-1} total DM (Austin, Chapter 2). The lower yields at Machakos are possibly the result of cooler average temperatures. Machakos is situated in highland Kenya at 1500 m in a cooler environment that reduces overall yield performance of leucaenas (Brewbaker *et al.*, 1985; Austin, Chapter 3).

Overall, psyllid damage in the trial was lower than from past infestations. It appears that psyllids are in equilibrium with other biotic factors such as biological controls and the environment. The highest psyllid damage ratings were taken in November 1993 and it is this month that should be used to compare to heavier infestations likely to be experienced in Africa in the first couple of years because of the lack of biological control organisms to keep the psyllid population in check. Environment also influences psyllid populations. The wet winter months are more conducive for heavy infestations. This is in contrast to Vivekanandan and Bandara (1990) who reported that psyllid infestations were lower during wet periods in Sri Lanka. The good psyllid resistance of *L. esculenta* and *L. pallida* selections came as no surprise. Both of these lines

exhibited superior resistance compared to *L. leucocephala* in other trials (Austin, Chapter 2; Austin *et al.*, 1995b; Glover, 1988). The higher damage levels of K953 compared to the other *L. pallida* entries may preclude its use in future breeding schemes.

The higher resistance of *L. diversifolia* K784 compared to K156 in this trial is confirmed by information from a concurrent forage trial at Waimanalo. While psyllid resistance differs between K784 and K156, biomass yields did not differ. Since K784 has demonstrated superior resistance it should be considered for future KX3 crosses. The advanced KX1, KX2 and KX3 selections showed intermediate psyllid resistance. This was also observed in Florida where F_2 hybrids were intermediate between the resistant *L. pallida* on one extreme and the susceptible *L. leucocephala* selections at the other (Austin *et al.*, 1995b). The better psyllid resistance of K565 compared to K636 during five of eight high psyllid damage periods may warrant its use in future breeding efforts to develop backcross progeny from interspecific F_1 hybrids like K748xK636. This approach will develop a forage variety with 75% *L. leucocephala* and 25% *L. pallida*.

Negative correlations between total biomass and psyllid resistance demonstrates the importance of psyllid resistance for lowland, tropical environments. Psyllid resistance is not an important objective for subtropical or highland

environments because the cooler temperatures and occasional frosts disrupt the psyllid life cycle (Austin *et al.*, 1995b). The high resistance combined with better seedling vigor of *L. pallida* are important agronomic considerations for developing *Leucaena* forage lines for lowland Africa.

CONCLUSIONS

Leucaena breeding scenarios for African conditions must include *L. pallida*. The *L. pallida* species offers good psyllid resistance and biomass yields over different environments. Psyllid resistance must be incorporated into *Leucaena* grown for forage in lowland, tropical environments. The *L. pallida* species also offers increased seedling vigor (Sorensson *et al.*, 1994) which will increase canopy closure and eliminate weed competition. Future breeding should focus on developing F_1 *L. pallida* x *L. leucocephala* for forage production in the low tropics, and *L. diversifolia* x *L. leucocephala* for highland environments.

CHAPTER 5

MINERAL NUTRIENT CONCENTRATIONS IN EDIBLE FORAGE FRACTIONS OF 20 *LEUCAENA* GENOTYPES AT WAIMANALO, HAWAII

ABSTRACT

Twenty *Leucaena* spp. genotypes grown at the Waimanalo Research Station, Waimanalo, Hawaii were analyzed for foliage nutrient composition. Edible forage fractions (leaf and small stems < 6 mm diam.) sampled from the fourth harvest were analyzed for P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn, B, and Al. Significant differences ($P<0.05$) were observed for K, Ca, Mg, Mn, Cu, and Zn. Variations for P, Na, Fe, B and Al were not significant. Nutrient means for all treatments were above National Research Council requirements for a 375 kg pregnant yearling heifer gaining 0.6 kg d^{-1}, with the exception of Na (0.03 %), Cu (6.7 mg kg^{-1}), and Zn (24.2 mg kg^{-1}).

INTRODUCTION

Leucaena forage is generally reported to contain adequate levels of macro- and micro nutrients required for ruminant diets. Much of the available information on mineral nutrient concentrations is limited to one species, *L. leucocephala* (Akbar and Gupta, 1984; Othman *et al.*, 1985). Very little is known about other species and

hybrids, with few exceptions (Austin *et al.*, 1990; Gupta *et al.*, 1986). The National Research Council (1984b) lists the following nutrient concentrations required for a 375 kg heifer gaining 0.6 kg d^{-1} : P (0.21 %), K (0.65 %), Ca (0.31 %), Mg (0.10 %), Na (0.1 %), Cu (8 mg kg^{-1}), Fe (50 mg kg^{-1}), Mn (40 mg kg^{-1}), Zn (30 mg kg^{-1}) and Co (0.1 mg kg^{-1}).

The purpose of this study was to compare the levels of 11 macro- and micronutrients in edible forage fractions of twenty promising *Leucaena* species and hybrids.

MATERIALS AND METHODS

The 20 *Leucaena* selections for this study (Table 5.1) were chosen from among 31 genotypes based on excellent performance or to fill in gaps in knowledge of the species. Seed from twenty *Leucaena* spp. selections were scarified by immersion for seven minutes in concentrated sulfuric acid, rinsed repeatedly in cool tap water, then allowed to soak in water overnight. Seed was inoculated with TAL 1145 rhizobium (Niftal Project, Maui, HI) and planted 20 March 1991 into biodegradable jiffy peat pots containing a sterile planting mix. Seedlings were watered three times daily in the greenhouse until ready to transplant.

Seedlings were transplanted 2 May 1991 into an Isohyperthermic Vertic Haplustol. The field was unfertilized but had a history of fertilization and previous maize production.

Table 5.1. Genotype and label of twenty *Leucaena* species and hybrids at Waimanalo, Hawaii.

Genotype	Label
L. leucocephala K636†	LEUC 636
L. leucocephala K500	LEUC 500
L. diversifolia (4n) K156	DIV4 156
L. diversifolia (2n) K749	DIV2 749
L. pallida K376	PALL 376
L. pallida K804	PALL 804
L. pallida K806 x K748	PALL HYB
L. collinsii K450	COLL 450
L. collinsii K905	COLL 905
L. esculenta K948	ESCU 948
L. esculenta K950	ESCU 950
L. greggii K857	GREG 857
L. lanceolata K952	LANC 952
L. multicapitula K880	MULT 880
L. salvadorensis K746	SALV 746
L. shannonii K769	SHAN 769
L. diversifolia K156 x *L. leucocephala* K636	DxL 1
L. pallida K806 x *L. leucocephala* K636	PxL 1
L. pallida K748 x *L. leucocephala* K636	PxL 2
L. pallida K806 x *L. diversifolia* K156	PxD 1

†K numbers based on University of Hawaii numbering system.

Seedlings were spaced 0.20 m apart in single rows 1 m apart producing a plant population of 50,000 plants ha^{-1}. Subsequent competition reduced this number to approximately 40,000 plants ha^{-1} by the time this study was initiated. Irrigation was applied weekly until the first harvest of 14 August 1991. Weeds were controlled within the row by hoeing and between rows by mowing.

Samples were taken for mineral analysis during the fourth harvest on 27 March 1992 from 2 replications when plant heights averaged 2.0 to 2.5 m. Eight plants were harvested intrarow (2 m linear distance) and cut to a height of 0.5 m to determine yield. Single plant subsamples were removed and stripped into edible forage (leaf and small stem < 6 mm diam.) and inedible fractions (remainder). Hand separated edible samples were placed in a kraft paper sack and dried under forced air at 60° C for 2 wk. Samples were analyzed for P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and Al in 1N HCl by the Agricultural Diagnostic Center, University of Hawaii on a Perkin-Elmer 6500 inductively-coupled plasma emission spectroscopic analyzer (Isaac and Jones, 1972). Boron was analyzed with the azomethine-H method (Wolf, 1974).

Data were analyzed as a randomized complete block (RCB) with two replications using the GLM procedure of SAS (1986). Mean separations were performed by Duncan-Waller multiple range test.

At the time of harvest, most plants were rapidly growing and none were flowering or fruiting. Only the *L. leucocephala* genotypes were under attack from psyllids (*Heteropsylla cubana* Crawford). Overall, psyllid populations were low during time of harvest with most of the *Leucaena* genotypes investigated having moderate to high psyllid resistance.

RESULTS AND DISCUSSION

Differences between *Leucaena* genotypes for macronutrients ($P<0.05$) were observed for K, Ca and Mg (Table 5.2). Phosphorous and Na did not differ and are presented without further analysis (Table 5.2). The means of the macronutrients were higher than required with the exception of Na. Sodium concentration of 0.03 % was much lower than the 0.1 % required for ruminant nutrition.

Differences ($P<0.05$) between the micronutrients were observed for Mn, Cu and Zn (Table 5.3). Manganese and Fe means were higher, and Cu and Zn means were lower than NRC requirements. Copper had a mean concentration of 6.7 compared to 8 mg kg^{-1}, while Zn was 24.2 compared to 30 mg kg^{-1} (Table 5.3).

Both B and Al concentrations did not differ between genotypes. Boron concentration averaged 52 mg kg^{-1}, while mean Al concentration was 58 mg kg^{-1} (Table 5.3).

Table 5.2. Macronutrient concentration (% DM) of twenty *Leucaena* spp. genotypes at Waimanalo, Hawaii.

Genotype	P	K	Ca	Mg	Na
LEUC 636	0.26 NS	2.5 a†	0.74 d	0.28 abcd	0.08
LEUC 500	0.26	2.4 ab	1.55 abc	0.31 ab	0.02
DIV4 156	0.27	2.0 abcde	0.98 bcd	0.21 cde	0.03
DIV2 749	0.33	2.2 abcd	1.32 abcd	0.24 bcde	0.04
PALL 376	0.28	2.2 abcd	0.85 cd	0.21 cde	0.02
PALL 804	0.32	2.0 abcde	0.86 dc	0.19 e	0.04
PALL HY	0.30	2.3 abc	0.91 bcd	0.19 e	0.02
COLL 450	0.29	1.8 bcdef	1.57 abc	0.22 cde	0.02
COLL 905	0.26	2.1 abcde	1.57 abc	0.21 cde	0.03
ESCU 948	0.34	1.8 bcdef	1.03 bcd	0.19 e	0.03
ESCU 950	0.25	1.5 def	0.97 bcd	0.16 e	0.02
GREG 857	0.28	1.3 f	1.17 bcd	0.29 abc	0.01
LANC 952	0.27	2.2 abcd	1.95 a	0.23 bcde	0.02
MULT 880	0.34	2.3 abc	1.00 bcd	0.22 cde	0.04
SALV 746	0.26	2.1 abcde	1.34 abcd	0.17 e	0.03
SHAN 769	0.27	2.1 abcde	1.65 ab	0.20 de	0.03
DxL 1	0.26	2.0 abcde	1.33 abcd	0.34 a	0.04
PxL 1	0.27	2.0 abcde	1.43 abcd	0.28 abcd	0.02
PxL 2	0.26	2.0 abcde	0.92 bcd	0.22 cde	0.03
PxD 1	0.28	1.5 ef	0.97 bcd	0.16 e	0.02
Mean	0.28	2.04	1.20	0.22	0.03
Std dev.	0.039	0.36	0.38	0.05	0.01
CV	13.3 %	13.1 %	24.6 %	16.7 %	44.0%
NRC‡	0.21	0.65	0.31	0.10	0.10

NS = not significant.
†means within column followed by different letters are different at ($P<0.05$) by Duncan Waller Mean separation.
‡NRC requirement for a 375 kg heifer gaining 0.6 kg d^{-1}.

Table 5.3. Micronutrient concentration (mg kg^{-1}) of twenty *Leucaena* spp. genotypes at Waimanalo, Hawaii.

Genotype	Mn	Fe	Cu	Zn
LEUC 636	50.5 bc†	240 NS	5.5 bcdef	25.5 abcd
LEUC 500	93.0 a	240	8.5 abc	27.5 abc
DIV4 156	51.5 bc	192	7.0 abcde	21.5 bcd
DIV2 749	48.0 bc	146	4.5 cdef	30.0 ab
PALL 376	44.0 bc	154	9.5 ab	25.5 abcd
PALL 804	44.5 bc	159	8.0 abcd	25.5 abcd
PALL HY	40.5 bc	129	7.5 abcde	27.0 abcd
COLL 450	41.0 bc	138	8.5 abc	23.0 abcd
COLL 905	36.5 bc	131	3.5 ef	18.0 cd
ESCU 948	55.5 bc	149	3.0 f	25.0 abcd
ESCU 950	40.0 bc	241	6.5 bcdef	31.5 a
GREG 857	30.5 c	73	4.5 cdef	21.0 bcd
LANC 952	51.5 bc	111	6.5 bcdef	20.0 cd
MULT 880	57.0 bc	217	8.5 abc	27.5 abc
SALV 746	42.5 bc	172	8.5 abc	21.0 bcd
SHAN 769	43.0 bc	116	4.0 def	17.5 d
DxL 1	61.0 b	185	9.0 ab	26.0 abcd
PxL 1	59.5 b	170	11.0 a	27.5 abc
PxL 2	38.0 bc	139	6.5 bcdef	23.0 abcd
PxD 1	60.0 b	117	4.5 cdef	21.5 bcd
Mean	49.4	161	6.7	24.2
Std. Dev.	15.5	57	2.5	4.5
CV	23.3 %	30 %	25.8 %	14.9 %
NRC‡	40.0	50	8.0	30.0

NS = not significant.
†means within column followed by different letters are significantly different ($P<0.05$) by Duncan's mean separation.
‡NRC requirement for a 375 kg heifer gaining 0.6 kg d^{-1}.

Table 5.4. Boron and Aluminum concentrations (mg kg^{-1}) of twenty Leucaena spp. genotypes at Waimanalo, Hawaii.

Genotype	B	Al
LEUC 636	61 NS	83 NS
LEUC 500	48	114
DIV4 156	58	36
DIV2 749	59	37
PALL 376	57	74
PALL 804	60	71
PALL HY	45	39
COLL 450	39	42
COLL 905	46	20
ESCU 948	63	36
ESCU 950	35	120
GREG 857	38	12
LANC 952	49	32
MULT 880	40	57
SALV 746	56	75
SHAN 769	65	37
DxL 1	55	86
PxL 1	57	82
PxL 2	50	27
PxD 1	67	33
Mean	52	58
Std. Dev.	12	38
CV	20	56

NS = not significant.

Mineral levels of edible *Leucaena* fractions reported herein, with the exception of Zn, are comparable to those previously reported. With few exceptions lines within species did not vary significantly in nutrient levels. Differences in Ca concentration were observed between *L. leucocephala* cultivars K636 and K500 (0.74 vs. 1.55 %, respectively) and in Cu concentration within *L. colinssii* K905 and K450 (3.5 vs 8.5 mg kg^{-1}, respectively). The basis for these differences is unclear.

The two *L. leucocephala* genotypes in this study (K636 and K500 cv. Cunningham) have superior yields worldwide, but were rarely superior to any other species or hybrids in nutrient concentrations. Potassium levels were higher than those of *L. esculenta*, *L. greggii* and *L. pallida* x *L. diversifolia* PxD 1. Magnesium levels were higher than those of *L. esculenta*, *L. salvadorensis* and two of the three *L. pallida* selections (K804 and PALL HYB). While there were statistical differences between mineral nutrient concentrations, it is unlikely that differences exist at the biological level. Levels of other minerals were not significantly different between *L. leucocephala* and other *Leucaena* selections.

Variability of mineral concentrations within lines was largely as expected with coefficients of variation (CV) around 20 %. CV values for P, K, Mg, and Zn were low (13.1

to 16.7 %). Sodium and and Al had very high CV values of 44 and 57 %.

CONCLUSION

Overall, all of the *Leucaena* species and hybrids involved in this study have acceptable concentrations of minerals to serve as forage for ruminant production. Average concentrations for six of the nine minerals tested exceeded the critical daily requirements listed by the National Research Council. The three minerals found in inadequate concentrations were Na (0.03 %), Cu (6.8 mg kg^{-1}), and Zn (30 mg kg^{-1}).

CHAPTER 6

YIELDS OF MIXED VERSUS PURE STANDS OF N-FIXING TREES AND *EUCALYPTUS GRANDIS*

ABSTRACT

Mixtures of nitrogen-fixing tree species may alleviate the need for N fertilizer in *Eucalyptus* plantations. Four species, *Eucalyptus grandis* Hill ex Maid. (EUC), *Leucaena leucocephala* (Lam.) de Wit. x *L. diversifolia* (Schlecht.) Benth. (KX3), *Paraserianthes falcataria* (L.) Nielson (FALC), and *Enterolobium cyclocarpum* (Jacq.) Griseb. (ENT) were harvested, samples dried, and wood and leaf biomass regressed vs. stem diameter and height to predict biomass. Stem diameter (DBH) was the best predictor of tree biomass, and height (HT) did not explain significant additional variation, except for KX3. Mean annual increment dry matter (DM) biomass of all plots at 4, 2 and 1 year rotations averaged 15.5, 15.6 and 8.2 Mg ha^{-1} yr^{-1}, respectively. One year rotations produced lower MAI ($P<0.001$) than either 2 and 4 year stands. *Paraserianthes falcataria* produced the highest MAI at 21.6 Mg ha^{-1} yr^{-1} over the range of rotation ages. The one-year yields of *Leucaena* KX3 were depressed by psyllids. Differences in height, DBH, leaf biomass, and leaf+small stem biomass of various pure and mixed-species

plots caused the allometric equations for total biomass to differ.

INTRODUCTION

The concept of short-rotation tree plantations was introduced in the 1970's (Szego and Kemp, 1973), and is particularly relevant in Hawaii since 90 % of its energy is imported (Crabb and Schubert, 1989). Electrical generation from biomass has a history in Hawaii owing to the use of sugarcane bagasse as an alternate energy supply (Schubert *et al.*, 1988). As of 1993, bagasse accounted for approximately 9-10 % of Hawaii's electrical needs (HSPA, 1993). Forestry has been suggested as an alternate use for lands being taken out of sugar production in Hawaii. Scraps from lumber or chipping could be converted into biofuel such as ethanol to offset the cost of imported fuel (Shleser, 1994). Since monocultural *Eucalyptus* plantations require N fertilizer for maximum production, mixtures of nitrogen-fixing tree species may alleviate the need for N fertilizer in short-rotation biomass schemes (DeBell *et al.*, 1985).

Eucalyptus growth is enhanced greatly by fertilizer amendments of N and to a lesser extent P (Cromer, 1971; Yost *et al.*, 1987; Whitesell *et al.*, 1988). Studies in Hawaii have shown that maximum growth was attained when approx. 200 kg N ha^{-1} yr^{-1} was used in three applications over a 15 month period (Yost *et al.*, 1987). Whitesell *et al.* (1992) found

that N fertilizer applications could be eliminated after the first year in mixed EUC/FALC trials on the Hamakua coast of Hawaii. Binkley *et al.* (1992) reported DM litterfall biomass in mixed EUC/FALC plots at around 13 Mg ha^{-1} yr^{-1} that recycles approximately 110-140 kg N and 6-7 kg P ha^{-1} yr^{-1}.

Average DM total biomass (leaves, stems and wood) yields in Hawaii reported as mean annual increment (MAI) are approximately 25 Mg ha^{-1} yr^{-1}. Yields of 6 year old *Eucalyptus grandis* Hill ex Maid. at 3363 tree ha^{-1} ranged from 18.4 to 26.2 Mg ha^{-1} yr^{-1} (Whitesell *et al.*, 1992). Four-year old *E. saligna* (Sm.) at 4444 tree ha^{-1} fertilized 3 times over a 15 month period with 50 g urea per application had MAI of 23.5 Mg ha^{-1} yr^{-1} at Akaka Falls, Hawaii (Yost *et al.*, 1987). DeBell *et al.* (1989) reported total biomass yield of pure 4 year old *E. saligna* at a population of 2500 trees ha^{-1} yielding 23.5 Mg ha^{-1} yr^{-1} MAI when fertilized with 40 kg N ha^{-1} yr^{-1}. Mixed plots of EUC/FALC in the same study had MAI of approximately 28 Mg ha^{-1} yr^{-1}. Pure stands of 6 year old *Eucalyptus* fertilized with 160 Kg N, 70 Kg P and 133 Kg K ha^{-1} yr^{-1} had MAI of 28.4 Mg ha^{-1} yr^{-1} (Dudley, 1990) planted at 10,000 trees ha^{-1}. Mean annual increment of 4, 5, 6 and 7 year old *Eucalyptus* ranged from 24.6 to 25.3 Mg ha^{-1} yr^{-1} (Whitesell *et al.*, 1992).

Biomass yields of nitrogen-fixing trees in Hawaii vary by site. Van den Beldt (1983) reported total DM biomass yields of *L. leucocephala* growing on four different Hawaiian

islands in a population of 10,000 trees ha^{-1} ranged from 12 to 32 MG ha^{-1} yr^{-1} and that wood yields of 4 year old *L. leucocephala* averaged 15 to 19 Mg ha^{-1} yr^{-1} at Waimanalo. Austin *et al.* (Chapter 2) reported total DM biomass yields of KX3 in a short-rotation forage trial at 22 Mg ha^{-1} yr^{-1} in Waimanalo. Total DM Yields of FALC in Hawaii ranged from 22 to 27 Mg ha^{-1} yr^{-1} (DeBell *et al.*, 1989; Whitesell *et al.*, 1992). Little published information concerning ENT yields was available anywhere. Mixed EUC/FALC plots grown in a 1:1 ratio yielded 26 Mg ha^{-1} yr^{-1} MAI after 4 years without N amendments (DeBell *et al.*, 1989). *Eucalyptus/Falcataria* plots in a 1:1 ratio on Hawaii yielded 17.6 Mg ha^{-1} yr^{-1} after 5.5 years, while EUC mixed with *Acacia melanoxylon* yielded 9.5 Mg ha^{-1} yr^{-1} (DeBell *et al.*, 1985).

A concern about mixed tree stands is that one species will eventually out-compete the other species nullifying the benefit of N-fixing litter. An example of this was reported from admixtures in Ka'u, Hawaii where FALC attained 6 m HT after 4 yr, while EUC attained a HT of 16 m (DeBell *et al.*, 1988). Mixed EUC/FALC trials planted at Chin Chuck, Hawaii in a 1:1 ratio at 2500 trees ha^{-1} developed a two-storied canopy after 4 years with EUC overtopping the FALC in height by more than 5.0 m (Whitesell *et al.*, 1992). At a mixed *Eucalyptus/Acacia* trial at Onomea, Hawaii, EUC had a mean HT of 13 m and DBH of 11 cm after 5.5 yr, while *A. melanoxylon* mean HT was 9 m and DBH of 8.5 cm (DeBell *et al.*, 1985).

Allometric equations for predicting total and woody biomass vary from site to site in Hawaii. Schubert *et al.* (1988) reported allometric equations for total DM biomass for 2 to 6 year old EUC and FALC trees as $Y=0.0694*DBH^{2.15}*HT^{0.31}$ and $Y=0.0362*DBH^{2.31}*HT^{0.36}$, respectively from the island of Hawaii. Allometry for total DM biomass of 1 to 4 year old EUC was $Y=0.135*DBH^{2.23}$ (Whitesell *et al.*, 1992) at Chin Chuck, Hawaii.

Eucalyptus mortality ranges widely in Hawaii. Whitesell *et al.* (1988) reported 39% mortality of *E. saligna* after 6 years. DeBell *et al.* (1989) reported low mortality rates ranging from 0 to 6 % after 4 years for both pure and mixed plots of EUC/FALC. DeBell and Whitesell (1988) reported 20 to 36% mortality of *E. saligna* in dense plantings of 5000 plants ha^{-1}. Schubert and Whitesell (1985) reported 38% mortality of *E. grandis* after 4.5 years at Kamae, Hawaii. Mortality of 5.5 yr old EUC was 12 % while mortality of EUC/FALC and *Eucalyptus/Acacia* in the same trial was 17 and 19 %, respectively (DeBell *et al.*, 1985). Mortality of the individual species in the mixed plots in this study was similar for *Acacia* and *Eucalyptus*, but differed for the EUC/FALC plots where 33% mortality was recorded for EUC but only 2% mortality was recorded for FALC (DeBell *et al.*, 1985).

This study was conducted to determine total biomass yields from mixed and pure stands of *Eucalyptus* and N-fixing

trees for use as an energy source for alleviating Hawaii's needs for imported fuel. Two main hypotheses will be examined notably will admixtures of N-fixing trees and *Eucalyptus* yield as well as pure stands without N fertilization, and will the allometry of trees be affected when grown in mixtures as opposed to pure stands.

MATERIALS AND METHODS

The Waimanalo research station is located at 21^0 20′ N, 158^0 20′ W with a mean elevation of 20 m above sea level. Precipitation ranges from 1000 to 1800 mm yr^{-1}. Mean annual temperature is 24.6°C. Temperature, rainfall and light are summarized for the period 1989-1993 in Appendix A. No fertilizer amendments or irrigation were applied to the soil before or during the experiment. Seedlings of four tree species *Eucalyptus grandis*, *Leucaena leucocephala* x *L. diversifolia* Benth. (KX3), *Paraserianthes falcataria* and *Enterolobium cyclocarpum* were transplanted 11 January and 14 March 1989. The experimental design was a split-plot with three replications and was based on four main plots representing harvest cycles of 1, 2, 3 and 4 year rotations. Seven treatments represented the subplots and included four monocultural plots and three mixed plots consisting of *E. grandis* + *Leucaena* KX3 (EUC+LEUC), *E. grandis* + *P.*

falcataria (EUC+FALC) and *E. grandis* + *E. cyclocarpum* (EUC+ENT).

Seedlings were planted at a 1 x 1.5 m spacing (6,600 plants ha^{-1}). Plots consisted of 5 rows spaced 1.5 m ten trees deep (50 trees $plot^{-1}$) representing a plot size of 75 m^2. Rows one and five were regarded as border rows and were not used in any of the biomass analysis. Harvest cycles were clustered in the whole plots to reduce light competition between harvest cycles. Trees in the mixed plots were planted alternately to provide a one to one ratio in tree species while maintaining 6,600 plants ha^{-1}.

The entire trial was cut back to 10 cm above ground height in December 1989 to initiate coppice regrowth. Soon after coppicing, the *E. grandis* suffered heavy mortality due to infection from *Phytopthora* spp. in some plots. Plots from replications one and two were combined into one replication leaving a total of two replications in order to provide representative pure and mixed *Eucalyptus* stands. The one-year plots were harvested in December 1990, 1991, 1992 and 1993. Two-year plots were cut in December 1991 and 1993, while the four-year plots were harvested in December 1993. Tree biomass of the one-year harvests from 1990 through 1992 and the two-year plots in 1991 were measured by harvesting all trees in the 3 interior rows, weighing, and subsampling for dry matter content of leaf, small stem and woody biomass. To maintain consistency with the 1993

harvest, the 3 one-year harvests and the 1991 two-year harvest had trees removed that were considered border trees from the final analysis of the data (trees numbered 1 and 10 were not analyzed).

A different approach was used for the 1993 harvest of the one, two and four year plots. Allometry was used to estimate total biomass yields using DBH and HT measurements taken prior to harvest. Measurements were taken from an area of 27 m^2 that represented the interior of each plot. Three subsamples were randomly selected from the pure plots, and four subsamples from the mixed plots (two trees per species). Samples were cut 10 cm above ground level, weighed, and stripped into leaf, stem and wood components. Dry matter of the wood fraction was determined from a wood core taken from the base of the plant weighed, and then dried at 70 ^0C for four weeks. The leaf and stem fractions were weighed, and subsampled and then dried for three weeks at 70 ^0C to determine their ratios to total biomass. The various plant components were then summed to produce a total DM weight of individual trees.

Total biomass was analyzed as a split-plot with treatment as the main plot and rotation age as the sub-plot using Proc GLM of SAS. If a significant treatment by rotation age interaction was detected the data were sorted by rotation age and analyzed as a RCB. The data were log transformed to correct for heterogenous variances generally

associated with diameter measurements. Mean separations were performed using orthogonal contrasts for rotation age. Duncan's multiple range test ($P<0.05$) was used to separate treatment means within rotation age.

Total biomass was calculated using allometric equations in Proc REG in SAS. A generalized form of the allometric model used was $\ln (W) = b_0 + \ln (DBH)$ where W is the dependent variable biomass, DBH is the predictor variable and b_0 the regression coefficient. Data were log-transformed prior to analysis. A slight bias of the coefficient caused by the regression of the log-transformed biomass was removed by multiplying the coefficient to a correction factor as the exponent (MSE/2) (Sprugel, 1983).

Analysis of variance using Proc GLM of SAS was used to detect interaction between treatment and ln DBH between pure and mixed plots. A significant interaction between treatment and DBH required that data for each treatment be analyzed separately to derive an allometric equation for predicting total biomass. Differences were based on type III sums of squares which calculates each term in the model as being entered into the model last. When interactions were not significant the interaction term was dropped and the model was run again to test treatment effects.

Mortality was measured in 1993 and analyzed as a RCB. If a significant treatment by age interaction was detected the data were analyzed on a yearly basis. Orthogonal

contrasts were used to separate the means of the various treatments.

RESULTS

Mortality

Harvesting on an annual basis led to high mortality of the EUC/FALC one year plots by the conclusion of the experiment. Mortality ranged from 0 to 100 % in both pure and mixed plots over the 3 rotation intervals (Table 6.1). *Enterolobium cyclocarpum* had the lowest overall mortality averaged in both mixed and pure plots with less than 1 %. *Eucalyptus grandis* had the highest overall mortality with 44 %, while FALC with 22 % and LEUC with 13 % were intermediate. *Eucalyptus grandis* had significantly higher mortality ($P<0.005$) than the nitrogen-fixing trees in the 2 and 4 year rotations. Pure plots had lower mortality ($P<0.05$) than mixed plots in years 1 and 2 (Table 6.1). Mixed EUC/ENT ($P<0.02$) and EUC/FALC plots had higher ($P<0.001$) mortality rates in year one than the EUC/LEUC plots (Table 6.1). A significant trt by age interaction for mortality ($P<0.001$) required that mortality be analyzed separately by harvest rotation.

Table 6.1. Percent mortality of pure and mixed N-fixing trees and *Eucalyptus grandis* plots† and orthogonal contrasts of mortality by rotation age in Hawaii.

	PURE PLOTS				MIXED PLOTS		
AGE	EUC	LEUC	FALC	ENT	EUC+LEUC	EUC+ENT	EUC+FALC
					Percent		
4	42	11	8	3	31(44+17)‡	20(39+0)	23(17+28)
2	36	6	6	0	14(11+17)	36(72+0)	28(50+6)
1	25	22	17	0	20(33+6)	36(72+0)	100(100+100)

	Mortality at Rotation Age		
Contrast	4	2	1
Pure vs. mixed plots	NS	*	***
EUC vs. NFT's	**	**	NS
LEUC vs. ENT and FALC	NS	NS	NS
FALC vs. ENT	NS	NS	NS
E+E vs. E+L and E+F	NS	NS	*
E+L vs. E+F	NS	NS	***

*, **, and *** and significant at $P<0.05$, 0.01 and 0.001 probability level, and NS = not significant.
†Treatments are *E. grandis* (EUC), *Leucaena leucocephala* x *L. diversifolia* (LEUC), *P. falcataria* (FALC), *E. cyclocarpum* (ENT), *E. grandis*+*Leucaena* (E+L), *E. grandis* +*E. cyclocarpum* (E+E), and *E. grandis*+*P. falcataria* (E+F).
‡Numbers in brackets denote mortality of *E. grandis* and the nitrogen-fixing tree, respectively.

Biomass yield

One year rotations averaged 8.2 Mg ha^{-1} yr^{-1} MAI, while 2 and 4 year harvest rotations averaged 15.6 and 15.5 Mg ha^{-1} yr^{-1} MAI, respectively (Table 6.2). The best yielding one year rotation was pure ENT with an average MAI over 4 seasons of 14.2 Mg ha^{-1} yr^{-1} (Table 6.2). *Paraserianthes falcataria* had the highest MAI for 2 year old stands with an average 21.6 Mg ha^{-1} yr^{-1} over 2 harvests and significantly differed ($P<0.05$) from ENT (Table 6.2). The 4 year old treatments did not differ and ranged from 14.4 to 19.6 Mg ha^{-1} yr^{-1} MAI. The MAI of pure EUC did not differ from the mixed plot yields over all harvest rotations (Table 6.2).

A significant rotation age ($P<0.001$) and treatment by rotation age ($P<0.01$) interaction required that the data be analyzed separably by rotation age (Table 6.3). One year rotations had significantly lower ($P<0.001$) total biomass yields than 2 and 4 year rotations (Table 6.3).

Diameter and height

Pure EUC heights differed ($P<0.01$) from mixed EUC by over 2.5 m after 4 yr (Table 6.4). Heights did not differ between pure EUC and the other mixed plots over any harvest rotation (Table 6.4). Two year old FALC was taller in pure stands ($P<0.001$) than in the mixed plots (Table 6.4).

Table 6.2. Total DM biomass yields and MAI† of pure and mixed tree plots over time in Hawaii.

Treatment‡	1990	1991	1992	1993	MAI
		-------- DM, Mg ha^{-1} --------			
1-year rotations					
E. grandis	7.1	8.5 ab§	8.2 ab	8.1 ab	8.0 bc
Leucaena KX3	12.5	6.7 b	7.8 ab	6.9 ab	8.5 bc
P. falcataria	9.5	3.5 b	4.7 b	3.2 ab	5.2 c
E. cyclocarpum	16.7	15.1 a	14.9 a	9.9 ab	14.2 a
EUC + LEUC	8.1	3.9 b	7.0 b	7.8 ab	6.7 bc
EUC + ENT	14.3	6.9 b	6.8 b	10.1 a	9.5 b
EUC + FALC	13.5	3.5 b	3.9 b	0.0 b	5.2 c
2-year rotations					
E. grandis		26.8 a		15.4 ab	10.6 b
Leucaena KX3		30.3 a		36.9 ab	16.8 ab
P. falcataria		35.9 a		50.5 a	21.6 a
E. cyclocarpum		26.5 a		16.0 ab	10.6 b
EUC + LEUC		31.6 a		43.8 ab	18.9 ab
EUC + ENT		31.7 a		39.3 ab	17.8 ab
EUC + FALC		40.2 a		12.0 b	13.1 ab
4-year rotations					
E. grandis				60.3 a	15.1 a
Leucaena KX3				63.4 a	15.8 a
P. falcataria				57.6 a	14.4 a
E. cyclocarpum				59.6 a	14.9 a
EUC + LEUC				53.6 a	13.4 a
EUC + ENT				78.5 a	19.6 a
EUC + FALC				60.2 a	15.0 a

†MAI (mean annual increment) is total biomass/rotation age.
‡Treatments in study are *Eucalyptus grandis*, *Leucaena leucocephala* x *L. diversifolia* KX3, *Paraserianthes falcataria*, *Enterolobium cyclocarpum*, *E. grandis* + *Leucaena* KX3 (EUC + LEUC), *E. grandis* + *E. cyclocarpum* (EUC + ENT), and *E. grandis* + *P. falcataria* (EUC + FALC).
§Different letters within columns are different ($P<0.05$, DMRT).

Table 6.3. Analysis of variance for mean annual increment of pure and mixed plots†, and contrasts for MAI at different rotation ages of pure and mixed stands in Hawaii. Type III sum of squares.

Source	df	SS	MS	F
Block	1	46894078	46894078	1.87 NS
Trt	6	151978411	25329735	1.01 NS
Error a	6	150737357	25122893	
Age	2	1304171813	652085907	28.17 ***
Trt*Age	12	708166643	59013887	2.55 **
Error b	70	1620450368	23149291	
Total	97	4063472702		

Rotation Age Contrast	df	SS	F
1 year vs. 2 and 4 year rotations	1	1304007824	44.90 ***
2 year vs. 4 year rotations	1	163991	0.01 NS

, * and NS are significant at P<0.01 and 0.001 and not significant, respectively.
†Pure plots consist of *E. grandis*, *L. leucocephala* x *L. diversifolia* KX3, *P. falcataria* and *E. cyclocarpum*, mixed plots were *E. grandis* + KX3, *E. grandis* + *P. falcataria*, and *E. grandis* + *E. cyclocarpum*.

Diameter at breast height was higher ($P<0.05$) for EUC in mixed plots with ENT than when grown alone in the 2 and 4 yr rotations (Table 6.4). *Enterolobium cyclocarpum* had significantly higher ($P<0.001$) DBH measurements in pure plots than in mixed plots at 4 yr, but smaller DBH ($P<0.05$) in the 2 yr rotation (Table 6.4). Likewise, FALC had smaller DBH ($P<0.001$) in the 2 yr mixed plot compared to the pure FALC stand.

Allometry

Four separate allometric equations were developed for EUC due to significant differences of the treatment by natural log of DBH interaction in both pure and mixed plots (Table 6.5).

The 3 nitrogen-fixing species each had a single equation to predict total biomass in both pure and mixed stands (Table 6.5). Based on non significance of the lnDBH*Trt interaction (Table 6.6).

A significant ln DBH by treatment interaction ($P<0.01$) for *E. grandis* in mixed and pure plots indicates differences in growth in each plot (Table 6.6). This is further evidenced by differences in the ln DBH by treatment interaction ($P<0.05$) detected for for both leaf and leaf and stem components (Table 6.7).

Table 6.4. Average DBH and HT and standard errors of pure and mixed N-fixing trees and *Eucalyptus grandis* plots at Waimanalo, Hawaii.

Tree Species	Age	DBH (cm) Pure	DBH (cm) Mixed	HT (m) Pure	HT (m) Mixed
Eucalyptus					
E. grandis	4	6.7±0.6	7.5±1.1 NS	9.9±0.5	10.6±0.6 NS
grown with	2	3.1±0.2	3.9±0.4 NS	6.9±0.3	6.8±0.5 NS
LEUC KX3	1	1.8±0.1	1.4±0.2 NS	4.0±0.2	3.8±0.3 NS
E. grandis	4	6.7±0.6	9.1±1.2 *	9.9±0.5	10.3±0.6 NS
grown with	2	3.1±0.2	5.3±1.8 *	6.9±0.3	7.4±1.3 NS
ENT	1	1.8±0.1	1.8±0.2 NS	4.0±0.2	4.5±0.4 NS
E. grandis	4	6.7±0.6	5.2±1.1 NS	9.9±0.5	7.0±1.1 **
grown with	2	3.1±0.2	3.0±0.5 NS	6.9±0.3	6.2±0.9 NS
FALC	1	1.8±0.1	No trees	4.0±0.2	No trees
Leucaena					
Leucaena KX3	4	4.7±0.3	3.8±0.3 NS	8.7±0.5	8.1±0.7 NS
grown with	2	3.3±0.2	3.6±0.3 NS	6.7±0.4	7.7±0.5 NS
EUC	1	2.0±0.1	2.3±0.2 NS	4.9±0.2	4.8±0.2 NS
Paraserianthes					
falcataria	4	4.5±0.3	4.0±0.4 NS	7.6±0.3	7.1±0.5 NS
grown with	2	4.5±0.3	2.3±0.2 ***	7.4±0.2	4.3±0.3 ***
EUC	1	1.4±0.1	No trees	2.5±0.2	No trees
Enterolobium					
Enterolobium	4	4.9±0.3	3.3±0.3 ***	7.2	6.2±0.4 N/A
grown with	2	2.3±0.1	3.0±0.2 *	5.5	5.5±0.3 N/A
EUC	1	2.0±0.1	2.0±0.1 NS	4.6	3.5±0.3 N/A

*, **, ***, significant at ($P<0.05$, 0.01 and 0.001), probability level and NS = not significant from tree species planted in pure plots.
†Pure *E. cyclocarpum* individual plant height were not measured.
N/A not applicable since *E. cyclocarpum* height was not measured.

Table 6.5. Allometric equations derived for tree species in pure and mixed plots combined over years for predicting total DM biomass production (W).

Tree Species	Allometric Equations	
E. grandis	$W = 0.19566505*DBH^{2.074636}$	$r^2=0.95$
E. grandis w/ *L. hybrid* KX3	$W = 0.27054144*DBH^{1.876222}$	$r^2=0.93$
E. grandis w/ *E. cyclocarpum*	$W = 0.17096012*DBH^{2.247670}$	$r^2=0.98$
E.grandis w/ *P. falcataria*	$W = 0.08101662*DBH^{2.543340}$	$r^2=0.99$
	$W = 0.02308314*DBH^{1.821956}*HT^{1.18465}$	$r^2=0.99$
L. hybrid KX3 alone & mixed	$W = 0.07812068*DBH^{2.763063}$	$r^2=0.96$
	$W = 0.01348279*DBH^{1.845689}*HT^{1.50254}$	$r^2=0.97$
E. cyclocarpum alone & mixed	$W = 0.05200877*DBH^{2.480858}$	$r^2=0.94$
P. falcataria alone & mixed	$W = 0.10682695*DBH^{2.248056}$	$r^2=0.88$

Table 6.6. Analysis of variance of four tree species for total biomass in SET 89-1 at Waimanalo, Hawaii.† Type III sum of squares.

		MS Values
Source	df	Eucalyptus
lnDBH	1	177.63***
TRT	3	0.63**
lnDBH*TRT	3	0.65**
Error	63	0.12
Total	70	

		MS Values		
Source	df	Leucaena	Falcataria	Enterolobium
lnDBH	1	90.86***	66.56***	62.92***
TRT	1	0.02 NS‡	0.01 NS	0.08 NS
lnDBH*TRT	1	0.09 NS	0.05 NS	0.01 NS
Error	37	0.13	0.28	0.10
Total	40			

, *, NS significant at P<0.01, 0.001, probability level and NS = not significant.
†Tree species were *E. grandis*, *Leucaena* hybrid KX3, *P. falcataria*, *E. cyclocarpum* grown in pure and mixed plots
‡If interaction term was NS then it was removed from the model and run again, TRT were still NS.

Table 6.7. Analysis of variance of 4 *E. grandis* treatments to determine the effect on wood and leaf (DM) fractions. Type III sum of squares.

		MS Values			
Source	df	Wood†	Leaf‡	Wood + Stem§	Leaf + Stem¶
lnDBH	1	234.99***	120.79***	198.43***	122.81***
TRT	3	0.04 NS	1.62*	0.39 NS	1.39**
lnDBH*TRT	3	0.52 NS	1.42*	0.46 NS	1.20*
Error	63	0.74	0.40	0.17	0.32
Total	70				

*, **, ***, significant at P<0.05, 0.01, 0.001, probability level and NS = not significant.
†Woody biomass of the tree (> 10 mm. dia.).
‡Leaf fraction of the tree.
§Wood and Stem fraction combined (small stems < 10 mm dia.).
¶Leaf and stem fraction combined (small stems < 10 mm dia.).

Differences in leaf and leaf and stem components of EUC at 1 and 2 year rotation ages when mixed with *Enterolobium cyclocarpum* affected overall growth of *Eucalyptus* in these plots (Table 6.8). Leaf and leaf + stem percentage as a function of total DM decreased with advanced rotation age for all trees, but when mixed with ENT the EUC showed significantly lower ($P<0.05$) percentages of these components (Table 6.8).

DISCUSSION

The EUC suffered from a fungal infection shortly after the plants were initially coppiced in 1989. Mortality was so high in some plots that it was decided to reduce the size of the experiment to 2 replications and 3 harvest rotations. This decision allowed flexibility to allocate enough plots in each block with adequate EUC populations so as not to affect the analysis of the experiment. The exception to this were the one and two-year old EUC/FALC plots. Overall mortality was similar to what Schubert and Whitesell (1985) reported for four yr old EUC trees in Hawaii.

Average MAI for the 2 and 4 year rotations in this trial was 15.5 Mg ha^{-1} yr^{-1} while in Hawaii average total tree biomass is approximately 25 Mg ha^{-1} yr^{-1} (DeBell *et al.* 1989; Whitesell *et al.* 1992). The best MAI was 25.2 Mg ha^{-1} yr^{-1} for FALC. Lower yields were due to high mortality rates and

Table 6.8. Leaf DM and Leaf + stem DM percent of total DM biomass between pure and mixed *E. grandis* plots over time in Hawaii.

Treatment	Age	Total DM leaf	Total DM Leaf + stem†
		--%--	--%--
E. grandis	4	9	17
	2	19	34
	1	39	59
E. grandis mixed with *L.* hybrid KX3	4	9 NS	17 NS
	2	17 NS	31 NS
	1	39 NS	59 NS
E. grandis mixed with *E. cyclocarpum*	4	11 NS	17 NS
	2	9 **	15 *
	1	25 *	37 *
E. grandis mixed with *P. falcataria*	4	12 NS	22 NS
	2	19 NS	32 NS
	1	N/A‡	N/A

*, **, significant at P<0.05 and).01 probability level and NS = not significant between EUC grown alone and EUC in mixed plots.
†Leaf and stem fraction combined (small stems < 10 mm dia.).
‡N/A not applicable since no harvest data were taken as a result of mortality.

low coppicing ability of EUC, and zero N-fertilizer application to the pure EUC. One year LEUC yields may havebeen affected by the *Leucaena* psyllid (*Heteropsylla cubana* Crawford) that feeds on young shoots of the plant commonly associated with frequent coppicing. Leucaena KX3 is a hybrid between *L. leucocephala* K636 and *L. diversifolia* K156, both parents and progeny are susceptible to the pest. *Paraserianthes falcataria* yields in years 2 and 4 are in sharp contrast to one-year rotations. In addition, the one and two year pure and mixed plots may have been affected by shading from the 4-year plots.

Average HT and DBH in this experiment were lower than those reported by Whitesell *et al.* (1992) for EUC and FALC grown alone and in mixed plots. Height of EUC and FALC averaged over both pure and mixed plots was 17 m and 13 m, respectively after 4 years compared to 8.5 m HT and 7.4 m for EUC and FALC in this study, respectively. Diameter at breast heights were also lower in this experiment averaging 6 and 4.3 cm at Waimanalo compared to 11.8 and 11 cm for EUC and FALC, respectively at Chin Chuck, Hawaii. When fertilized with 50 kg N ha^{-1} yr^{-1} four year old EUC at Hilo, Hawaii averaged 13.6 m HT and 8.6 cm DBH (Whitesell *et al.*, 1988).

Differences in leaf ($P<0.05$) and leaf + stem ($P<0.05$) components between pure and mixed EUC were responsible for developing separate allometric equations (Table 6.5).

Variations of leaf, and leaf+stem yields of the EUC resulted in lower percentages ($P<0.05$) of these components in both 1 and 2 year rotations of EUC+ENT (Table 6.8).

The differences in EUC morphology between pure and mixed stands and the differences between treatments over rotation age is due to different growth patterns of the nitrogen-fixing trees. The lower leaf+stem ratio of EUC in EUC+ENT is due to the high coppicing rate and dense canopy characteristics of ENT. Two-year old ENT had an average of 3.1 stems plant^{-1} compared to EUC with 1.4. One-year ENT had an average of 5.8 stems, while EUC had 3.8. Competition for light was so severe in these environments that the remaining EUC was unable to compete. Frequent coppice intervals favor the ENT over the EUC. The 4 year mixed EUC (1 stem plant^{-1}) + ENT (3.1 stems plant^{-1}) ratio were not different because they were coppiced a single time at the beginning of the experiment.

CONCLUSIONS

Total biomass yields were lower than earlier reports from the island of Hawaii for this trial due to several biological constraints. High mortality, psyllid pressure and differences in growth habit all contributed to lower than expected yields. Morphological effects on *Eucalyptus grandis* through reduction in leaf and leaf + stem biomass required separate allometric equations to determine biomass

yield. The significant effect on EUC allometry is an important finding and must be considered when determining appropriate admixtures between N-fixing and non N-fixing trees.

CHAPTER 7

HERITABILITY OF PSYLLID RESISTANCE AND VIGOR IN ADVANCED PROGENIES OF KX2 AND KX3 *LEUCAENA* HYBRIDS

ABSTRACT

Information concerning the heritability of psyllid resistance and vigor of *Leucaena* spp. is limited. This study was conducted to determine their heritability in advanced generation progenies from KX2 (*L. pallida* x *L. leucocephala*) and KX3 (*L. diversifolia* x *L. leucocephala*), and test if psyllid resistance and forage vigor are related. A total of 27 open-pollinated (OP) and 12 self-pollinated (SELF) F_4 KX2, and 19 SELF F_3 KX3 families were grown for 2 years in Hawaii. Psyllid damage and vigor were rated empirically using scales of 1-9 and 1-6, respectively. Broad-sense heritabilities (h^2_b) and genetic gain (G) were determined. For psyllid damage, KX2 OP had h^2_b of 0.37, KX2 SELF had h^2_b of 0.90 and KX3 SELF had h^2_b of 0.65. The h^2_b of vigor for KX2 OP was 0.26, KX2 SELF was 0.86 and KX3 SELF was 0.70. Genetic gain (G) for psyllid damage of KX2 OP was 0.20 units per cycle, of KX2 SELF was 0.09 and of KX3 SELF was 0.12. Gains for vigor were 0.11, 0.08 and 0.20, respectively. The conclusion of the experiment, psyllid damage means for KX2 OP and KX2 SELF progenies compared

against the entire F_3 population were 1.49 and 1.76 vs. 1.74. Vigor means were 4.54 and 4.68 vs. 3.67, respectively. Average psyllid damage and vigor ratings for KX3 SELF were 2.91 and 4.19, respectively. Selection for psyllid damage and vigor were negatively correlated for KX2 OP ($r= -0.22$, $P<0.008$), KX2 SELF ($r = -0.54$, $P<0.001$) and KX3 SELF ($r = -0.52$, $P<0.001$) which indicates that both traits can be selected for simultaneously. Approximately 75 % of the test material was rogued by the end of the trial, and the remaining trees are being used for seed production.

INTRODUCTION

The hybrid KX2 (*L. pallida* x *L. leucocephala*) has favorable qualities for lowland, tropical environments due to good psyllid resistance and biomass production (Wheeler and Brewbaker, 1989). This hybrid has shown high resistance to psyllids (*Heteropsylla cubana* Crawford) in both Florida and Hawaii (Austin *et al.*, 1995b; Wheeler and Brewbaker, 1989), with resistance coming from the *L. pallida* parent. *Leucaena pallida* hybrids and species also have improved seedling vigor (Sorensson *et al.*, 1994), and better cold tolerance (as noted in Chapter 3) than *L. leucocephala*, important factors for alleviating weed competition and for expanding the range of leucaenas. In the absence of psyllid pressure, *L. leucocephala* lines produce high biomass and forage yields. Thirty Mg of edible forage ha^{-1} yr^{-1} has been

recorded in Hawaii (Brewbaker *et al.*, 1972) prior to the psyllid invasion of 1984. *Leucaena leucocephala* also has the highest digestibility tested to date within the genus (Austin *et al.*, 1995a).

Leucaena leucocephala (2n=104) is self-compatible, while *L. pallida* (2n=104) is self-incompatible (Brewbaker, 1982; Sorensson and Brewbaker, 1994). KX2 was largely self-incompatible in the F_1 generation but segregated self-fertile trees. Self-incompatibility tends to become less frequent in later generations of such hybrids (Brewbaker, 1954).

In contrast to KX2, the KX3 progeny of *L. diversifolia* x *L. leucocephala* are self-pollinating. A simple pedigree method of breeding can be used to develop homogeneous progeny which express excellent growth under lowland and highland environments.

Increased cold tolerance is desired to extend the range of *L. leucocephala* from relatively narrow tropical lowland ecozones to higher elevations. The interspecific hybrids of *L. diversifolia* and *L. pallida* with *L. leucocephala* showed superior cold tolerance at an elevation of 900 m at Mealani, Hawaii (Brewbaker *et al.*, 1988). Growth rates of 4.5 m yr^{-1} were recorded for KX3 over a two year period at Mealani (Brewbaker *et al.*, 1990).

Progeny testing can provide estimates of relative genetic value of parents based on the performance of their

offspring (Allard, 1960). Robertson (1957) found that genetic gain in animals was maximized by testing as few as 5 offspring per family, and encouraged more extensive testing of families. However, Cotterill and James (1984) working with *Pinus radiata* found that 10 to 20 outcrossed offspring per family were sufficient to evaluate each family.

Partitioning of the phenotypic variance into three components, genotype (G), environment (E), and genotype*environment (GE) allows for estimation of the relative importance of these three effects on the phenotype (Nyquist, 1991). Broad-sense heritability (h^2_b) is the ratio of the variance of the genotypic values in the population to the phenotypic variance (σ^2_G/σ^2_P) and is that proportion of the phenotypic variance that is determined by the genotype (Nyquist, 1991). While h^2_b can be assessed by parent-offspring regression, narrow-sense heritability (h^2_n) - a ratio of additive to phenotypic variance requires specifically designed progeny trials not available in the present study.

The truncating of populations by selection had the effect of increasing h^2_b for diameter in *Pinus radiata* from 0.18 to 0.27 (Matheson and Raymond, 1984), enhancing genetic advance. While roguing trees that perform poorly will not change the heritability of a particular trait, selected parents may have different heritabilities than the population at large.

Genetic gains (G) can be estimated with the formula G=ish (Brewbaker, 1994), where i = the intensity of selection, s = the standard deviation of the phenotype and h = the narrow-sense heritability of the population. An important component of this equation is h and it should be based on h^2_n since it reflects a measure of the additive variance of the trait. If one selects parents with outstanding characteristics, the intensity of selection (i) increases, and genetic improvement can be accomplished even if the heritability or phenotypic variances are thus low. The potential genetic improvement through breeding is the product of the heritability of the trait, the intensity of selection, and the amount of phenotypic variation of the population for the selected trait (Brewbaker, 1994).

Plants show three types of resistance to insects -- nonpreference, antibiosis and tolerance (Zobel and Talbert, 1984). *Leucaena leucocephala* genotypes K636 and K584 have better psyllid tolerance than other genotypes within this species (Sorensson and Brewbaker, 1986; Wheeler, 1988). High psyllid resistance can be found in several other *Leucaena* species such as *L. pallida*, *L. collinsii* and *L. esculenta* (Brewbaker *et al.*, 1989). Morphological characteristics such as small leaflet size was not correlated (Sorensson, 1989), while mucilage production (Sorensson and Brewbaker, 1986) appears to be correlated with resistance. Pan (1989) reported a normal distribution

of psyllid resistance in F_2 KX3 populations, and concluded that mechanisms for resistance are oligo- or poly-genic. Blada (1980) found broad-sense heritabilities of insect resistance in larch clones *Larix decidua* Mill. and *L. leptolepis* (S. & Z.) attacked by *Adelges laricis* Vall. to range from 0.41 to 0.77. Expected gains (G) ranged from 7.9 to 15.1 % over three environments.

In slash pine (*Pinus elliottii*), heritability of growth as a function of volume was only 8 % at age 5 (Hodge and White, 1992). Dean *et al.* (1986) reported heritabilities ranging from 14 to 49 % for empirical estimates of form in *Pinus caribaea* var. *hondurensis* form (i.e. stem straightness, branch diameter, and branch angle).

The objective of this experiment was to select for high psyllid resistance and good forage vigor of the F_4 progeny in KX2 and KX3 populations. We hypothesize that psyllid resistance is simply inherited and that psyllid resistance is correlated to vigor.

MATERIALS AND METHODS

KX2 OP and SELF

The sequence of rating, coppicing and roguing of KX2 OP, KX2 SELF and KX3 advanced progenies is presented in Table 7.1.

Table 7.1. Time line for open pollinated and selfed KX2 and KX3 advanced progenies at Waimanalo, Hawaii.

Activity	Date	Plant Status
Transplanted:	May 21, 1992	Hardened seedlings
First Rating:	July 16, 1992	Seedlings
Second Rating:	Aug. 20, 1992	Seedlings
Third Rating:	Oct. 20, 1992	Seedlings
First Rogue:	Jan. 30, 1993	Trees, pollination
Second Rogue:	Apr. 21, 1993	Trees, seed collected.
First Coppice:	July 27, 1993	50 cm above ground
Fourth Rating:	Aug. 23, 1993	Coppice regrowth
Third Rogue:	Aug. 24, 1993	
Second Coppice:	Feb. 6, 1994	50 cm above ground
Fifth score:	Mar. 28, 1994	Coppice regrowth, Parents rated at this time
Fourth Rogue:	Apr. 14, 1994	
Sixth Score:	May 1, 1994	Coppice regrowth, Parents also rated this time

The KX2 families under study originated from crosses initiated by F.J. Pan (Ph.D. Dissertation, 1985) of K376 x K8 (*L. pallida* x *L. leucocephala*). Open pollinated seeds were collected from 58 KX2 trees, a highly selected surviving sample from 1500 F_3 trees in experiment SET 89-6 in 1991. Twenty-two of the 58 trees also produced selfed seeds in varying quantities (Table 7.2). Superior trees were identified previously by Brewbaker and Wheeler (unpublished) based largely on psyllid resistance, vigor and coppicability.

Seeds were scarified in boiling water, drained and let stand overnight in tepid water. Seeds were inoculated with rhizobium and planted into dibble tubes containing peat on 4 Feb. 1992. Poor germination resulted in the loss of 18 lines, while some other lines produced only a few trees (Table 7.2).

A total of 27 open pollinated and 12 self pollinated lines were transplanted into 2 replications on 21 May 1992 into an unfertilized Typic Vertic Haplustoll. Each plot contained from 2 to 20 trees planted in double rows at 0.25 m spacing intrarow and 2 m spacing interrow. Favorable rainfall immediately after planting (40 mm in 3 days) helped the plants to establish.

Plants were evaluated for psyllid resistance and vigor on 6 separate occasions from 1992 to 1994 (Table 7.1).

Table 7.2. KX2 Seed Collection of open-pollinated OP and selfed SELF lines and the number of seedlings ready for transplanting in 1992.

Tree #	Selfed seed	Seedling# (SELF)	Trans. (SELF)	Seedling # (OP)	Trans. (OP)
1-3	190	48	Y	37	Y
1-5	86	0		12	Y
1-8	19	0		1	
2-4	67	16	Y	5	Y
2-9				8	Y
3-3	5	0		40	Y
3-5				20	Y
3-6	21	4	Y	43	Y
3-9	58	0		46	Y
3-22	13	0		0	
3-26	31	18	Y	23	Y
3-32				26	Y
3-33	42	0		3	
3-36				9	Y
4-1	3	0		0	
4-7				37	Y
4-13				5	Y
5-3	56	35	Y	40	Y
5-9	35	1		16	Y
5-20	40	4	Y	41	Y
5-33	42	0		17	Y
5-36	39	6	Y	50	Y
6-2	20	9	Y	4	Y
6-9	398	48	Y	5	Y
6-15	228	44	Y	38	Y
6-16	10	0		0	
6-20	55	18		46	Y
6-28				5	Y
6-34	18	4	Y	42	Y
6-38				12	Y
7-5	60	43	Y	0	
7-8				16	Y
7-13				9	Y
33 LINES	22 LINES		12		27

Psyllid resistance was rated on an empirical scale of 1 to 9 (1=no damage, 9=complete defoliation) as reported by Glover (1987). Since destructive sampling of plants to determine vigor would affect growth a simple scale was developed to determine vigor using an empirical scale of 1 to 6 (1=poor, 6=excellent) and was based on plant height, number of shoots, and leafiness of plants.

Seedlings were evaluated three times on 16 July, 20 August and 18 October 1992. The first and second roguings occurred on 30 January, and 21 April 1993 and were based on data from these first three evaluations. Seedlings were rogued by cutting the plant stem at ground level. The entire trial was coppiced to 0.5 m height on 27 July 1993 and the regrowth was rated on 24 August 1993 at which time the third rogue occurred based on data from these scores. The trial was then allowed to grow and was coppiced again on 6 February 1994. The parent F_3 trees in SET 89-6 were also coppiced at this time. Both the parents and the progeny were rated for psyllid damage and vigor on 28 March 1994. The fourth rogue occurred on 14 April 1994 and was based predominantly on vigor. Psyllid damage ratings were low in March 1994 and both trials were rescored for both traits 1 May 1994 on rapidly growing plants.

Analysis of variance and Duncan's multiple range tests were used for mean separation. The KX2 OP families means were analyzed by between-within analysis of variance to

provide estimates of broad-sense heritability for psyllid damage and vigor as h^2 = Vg/Vp where Vg = (Variance of the families - error variance)/reps. Offspring of KX2 SELF lines were assumed to be fully selfed with an inbreeding coefficient = 1. Broad-sense heritability for KX2 SELF means was also estimated by h^2 = Vg/Vp. In order to estimate heritability, data prior to the first rogue on 30 Jan 1993 had to be used. For psyllid damage, ratings taken from 16 July 1992 were used since these represented the highest psyllid damage scores. For vigor, ratings from 18 Oct 1992 were used since this represented 5 months growth. Gains related to selection were calculated as G = ish where i =selection intensity, s = the standard deviation of the phenotype, and h = the heritability (Becker, 1992; Brewbaker, 1994). Parent trees were ranked from 1 to 27 and were based on the plot means for psyllid damage and vigor, with good rankings indicating top performance (Appendix D).

KX3

Open pollinated seeds were collected from 20 F_2 trees of KX3 (K156 x K636) from Waimanalo and elsewhere on Oahu in 1991 (Table 7.3). Two lines, numbered 91-1 and 91-3 were collected to serve as checks in the trial. Seed was scarified and inoculated in the same manner as the KX2 lines. Seeds were planted into dibble tubes containing peat

Table 7.3. KX3 seed collection of open-pollinated lines and the number of seedlings ready for transplanting in 1992.

Tree #	Collected Seed (number)	Seedling number (out of 100 seeds)	Location collected
91-1	371	48	K field
91-2	153	25	K field
91-3	658	46	K field
91-4	67	0	K field
91-5	469	35	K field
91-6	310	39	K field
91-7	40	34	K field
91-8	730	44	K field
91-9	107	43	K field
91-10	148	48	K field
91-11	739	42	K field
91-12	150	45	K field
91-13	145	50	K field
91-14	685	24	K field
91-15	51	9	I field
91-16	207	9	K field
91-17	193	32	Kailua
91-18	201	37	Kailua
91-19	120	47	Seed Facility
91-20	120	45	Seed Facility
20 LINES		19 LINES	

on 4 Feb. 1992. Poor germination resulted in the loss of 1line, while three other lines produced fewer than the required 40 trees (Table 7.3). Nineteen lines were transplanted into 2 replications on 21 May 1992 into an unfertilized Typic Vertic Haplustoll. Each plot contained anywhere from 4 to 20 trees. Favorable rainfall immediately after planting (40 mm in 3 days) helped establishment. Experimental methods were otherwise identical to those of the KX2 progenies.

Analysis of variance and Duncan's mean separation were used to test the means. Analysis of heritability was based on between-within family mean analysis. Genetic gains were derived from G = ish. Rankings were based on psyllid damage and vigor averages. Good numeric rankings i.e 1>2>3 indicate top performance (Appendix D).

RESULTS

Seed collection and germination

Seeds from 58 of a total 184 (32 %) parental F_3 trees of KX2 in SET 89-6 were collected for this experiment. Twenty-two of the initial 58 (38 %) parent trees selected could be self-pollinated while the remaining 36 trees could not set selfed seed. 21 families (36 %) were removed from the experiment due to poor germination leaving 37 families that produced seedlings. Ten families produced very few seedlings and were omitted from the experiment resulting in

27 OP families and 12 SELF families for the trial. Only 1 selfed line (Family 7-5) does not also appear in the OP trial.

KX2 OP

A total of 597 trees were planted and of these 74 % or 425 were rogued by the end of the experiment (Table 7.4). The best lines had the lowest number of progeny rogued, averaging 53 %. Mortality was less than 4 % of the total number of trees planted (Table 7.4).

The best open-pollinated families were trees designated 5-36, 3-32, and 7-13. Progenies from these three lines had both high psyllid resistance and vigor over all rating periods. Differences ($P<0.001$) for psyllid damage were detected between KX2 OP F_4 progenies after two months (Table 7.5). When family means were analyzed a significant difference ($P<0.05$) between families for psyllid damage was detected (Table 7.5). From this analysis broad-sense heritability for psyllid damage of KX2 OP was 0.371 and genetic gain was 0.206 (Table 7.5).

After two years the parental F_3 KX2 population of 184 trees had a mean psyllid damage rating of 1.74, the 27 parent trees selected from that population was 1.62, and the remaining F_4 KX2 OP progeny had a mean psyllid damage rating of 1.49 (Figure 7.1).

Table 7.4. Number of KX2 OP trees rogued over time in SET 92-3 at Waimanalo, Hawaii.

Tree #	Start	Died	Sub-Total	Rogue Number 1	2	3	4	Rogue Total	%
3-3	40	3	37	11	9	10	3	33	89
3-6	40	0	40	12	9	6	3	30	75
3-9	40	1	39	16	8	3	4	31	79
5-3	40	0	40	14	11	5	3	33	83
5-20	40	1	39	11	6	6	6	29	74
5-36	40	3	37	11	8	4	1	24	65
6-20	40	1	39	14	10	7	4	35	90
6-34	41	0	41	11	10	6	6	33	80
6-15	36	0	36	4	12	6	1	23	64
4-7	35	1	34	10	8	6	3	27	79
1-3	36	0	36	12	12	6	3	33	92
3-32	27	1	26	6	5	0	2	13	50
3-5	20	5	15	5	2	2	2	11	73
3-26	22	0	22	4	5	4	2	15	68
5-33	10	0	10	2	3	3	2	10	100
7-8	16	0	16	1	4	4	1	10	63
5-9	18	2	16	3	3	2	2	10	63
2-4	4	0	4	0	0	2	1	3	75
4-13	4	0	4	0	1	2	0	3	75
6-2	4	0	4	0	0	1	1	2	50
6-9	4	0	4	0	2	0	0	2	50
6-28	4	0	4	0	0	2	1	3	75
7-13	8	0	8	0	2	0	1	3	38
2-9	4	0	4	0	1	0	0	1	25
6-38	12	4	8	0	0	0	1	1	13
3-36	2	1	1	0	0	0	0	0	00
1-5	10	0	10	0	2	2	3	7	70
TOTALS									
27	597	23	574	148	133	90	54	425	74

Table 7.5. Analysis of variance and estimates of heritability and genetic gain for psyllid resistance of KX2 OP family means from July 16, 1992.

ANOVA

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	3.213	3.213	3.73 NS	3.86
Families	26	234.947	9.036	10.49***	1.55
Error	567	488.636	0.861		
Total	594	730.539			

ANOVA of Family Means of all progenies replicated twice

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.823	0.823	1.83 NS	4.28
Families	23	22.542	0.980	2.18 *	2.00
Error	23	10.337	0.449		
Total	47	33.703			

Components of Variance

Vfamily = (MSfam-MSerror)/rep = 0.270
Ve = 0.450
Vp = 0.710

Broad-sense heritability for psyllid resistance = 0.371

Genetic Gain = i*s*h

i = 25% = 1.242
s= 0.449
h = 0.371

Gain (G) = 0.206

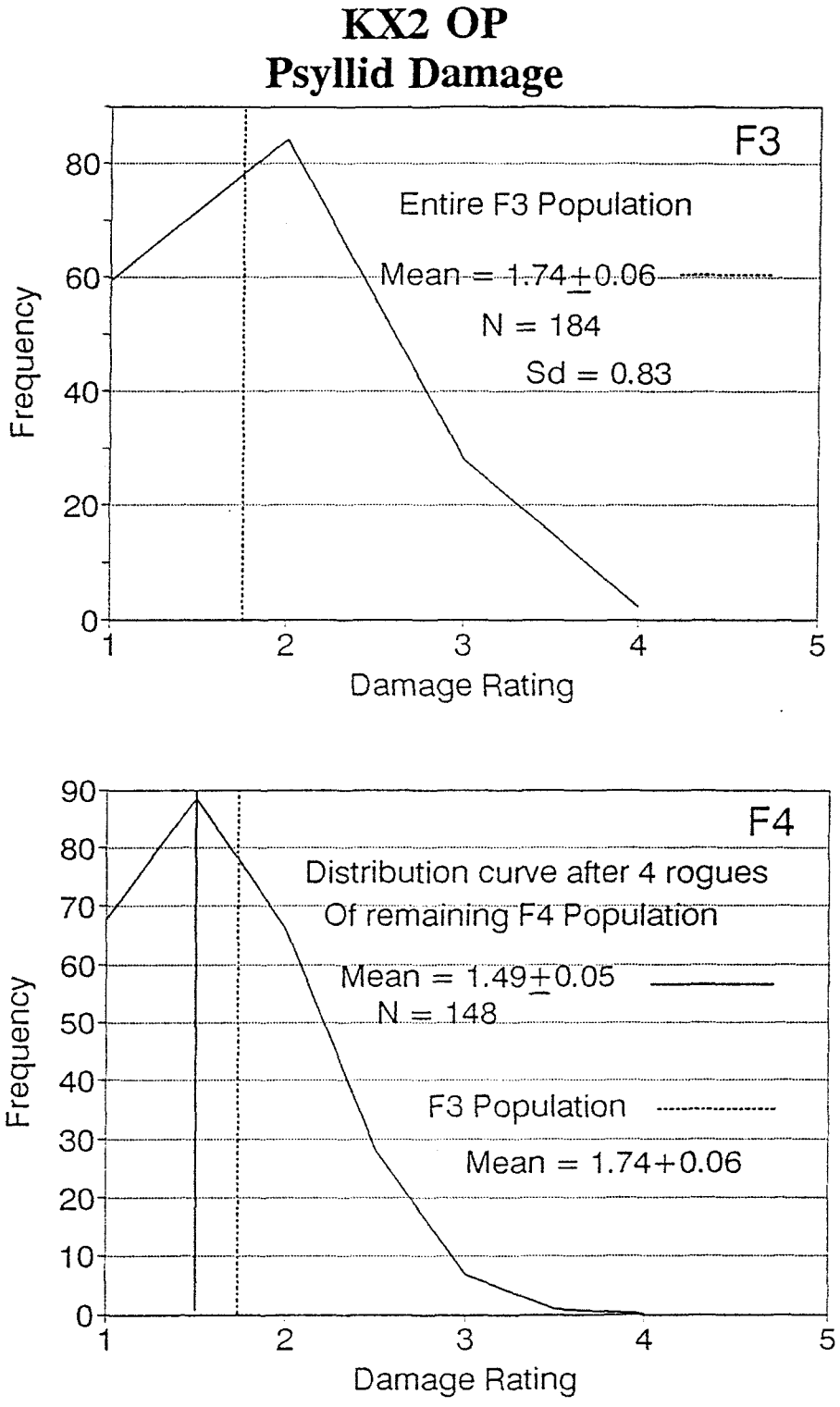

Figure 7.1. Variance distribution of F_3 and F_4 Leucaena KX2 open-pollinated (OP) lines for psyllid damage taken May, 1994. Psyllid damage (1=no damage, 9= complete defoliation). Sd = standard deviation.

Vigor ratings were also better for the F_4 progeny. The 184 parental trees in the F_3 population had a mean vigor rating of 3.67, while the 27 selected parents was 4.68 and the F_4 progeny had a mean vigor rating of 4.54 (Figure 7.2). Unlike psyllid resistance, vigor ratings from 18 Oct 1992 were significant ($P<0.001$) using analysis of variance for the entire data set but not for family means (Table 7.6). Broad-sense heritability of the family means for vigor was 0.255, while expected gain (G) was 0.11 (Table 7.6).

Psyllid damage and vigor were significantly and negatively correlated at the conclusion of the trial and was $r = -0.22$, $P<0.008$.

KX2 SELF

Of 248 trees planted 72 % or 167 trees were rogued (Table 7.7). In comparison, only 63 % of the top four lines were rogued (Table 7.7). Mortality was 6 % in this experiment. The best lines were from progenies coming from parent trees 5-36, 6-9, 6-15 and 7-5, progenies from these lines had high psyllid resistance and good vigor over all observations.

Differences for psyllid damage were significant ($P<0.001$) between families using analysis of variance for both the entire data set and family means (Table 7.8). Broad-sense heritability of the family means for psyllid

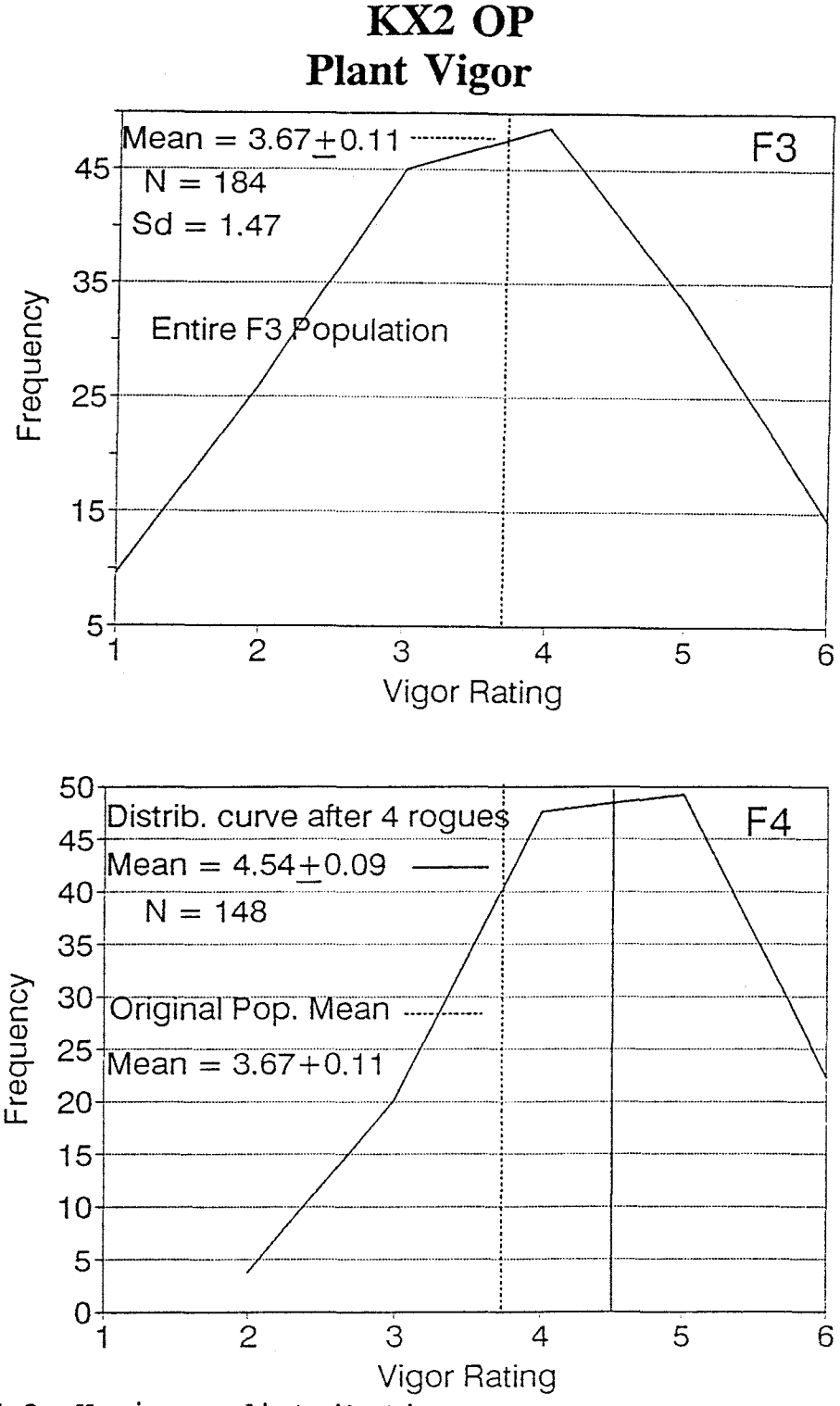

Figure 7.2. Variance distribution of F_3 and F_4 Leucaena KX2 open-pollinated (OP) lines for vigor taken May, 1994. Vigor ratings (1= low, 6= very high). Sd = standard deviation.

Table 7.6. Analysis of variance and estimates of heritability and genetic gain for vigor of KX2 OP family means from Oct 18, 1992.

ANOVA

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	2.975	2.975	3.01 NS	3.86
Families	26	123.109	4.734	4.79***	1.55
Error	556	549.359	0.988		
Total	583	676.722			

ANOVA of Family Means of all progenies replicated twice

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.130	0.130	0.37 NS	4.28
Families	23	13.638	0.593	1.69 NS	2.00
Error	23	8.086	0.352		
Total	47	21.854			

Components of Variance

Vfam = (0.593-0.352)/2 = 0.120
Ve = 0.352
Vp = 0.472

Broad-sense heritability for plant vigor = 0.255

Genetic Gain = i*s*h

i = 25% = 1.242
s = 0.352
h = 0.255

Gain (G) = 0.111

Table 7.7. Number of KX2 SELF trees rogued over time in SET 92-3 at Waimanalo, Hawaii.

Tree #	Start	Died	Sub-Total	Rogue Number 1	2	3	4	Rogue Total	%
1-3	39	3	37	11	8	6	5	30	81
6-9	40	4	36	8	12	3	0	23	64
6-15	40	3	37	11	10	2	3	26	70
7-5	40	0	40	7	15	4	2	28	70
5-3	32	0	32	8	10	2	6	26	81
6-2	9	0	9	0	3	4	1	8	89
2-4	15	0	15	1	4	4	2	11	73
3-26	17	4	13	1	2	4	2	9	69
3-6	4	2	2	0	0	2	0	2	100
5-36	4	0	4	0	0	2	0	2	50
5-20	4	0	4	0	0	1	0	1	25
6-34	4	0	4	0	1	0	0	1	25
TOTALS									
12	248	15	233	47	65	34	21	167	72

resistance was 0.903 and the expected gain (G) was 0.09 (Table 7.8).

Mean psyllid damage of the parent F_3 population was 1.74, the parents selected from that population 1.55, and the F_4 SELF progeny 1.76 (Figure 7.3).

Vigor ratings also differed ($P<0.001$) when analyzed with analysis of variance (Table 7.9). Broad-sense heritability for family means of vigor was 0.90 and the expected gain was 0.08 (Table 7.9).

Vigor ratings after 2 years were 3.67 for the entire F_3 population, 4.68 for the selected F_3 parents, and 4.41 for the F_4 SELF progeny (Figure 7.4).

Psyllid damage and vigor were significantly ($P<0.02$) and negatively correlated at the conclusion of the trial and was $r = -0.54$, $P<0.001$.

The F_4 KX2 OP progeny had a lower psyllid damage average (1.49) than the previous F_3 population (1.74) and higher vigor rating (4.54) than the F_3 at 3.67 at the conclusion of the experiment (Table 7.10). The KX2 SELF F_4 progenies also had higher vigor scores than the F_3 population with 4.41 versus 3.67. Psyllid damage ratings were not different however, with both populations averaging 1.76 versus 1.74, respectively (Table 7.10). The coefficient of variation (CV) for KX2 OP, KX2 SELF and the F_3 population differed for both traits and was lowest for

Table 7.8. Analysis of variance and estimates of heritability and genetic gain for psyllid resistance of KX2 SELF family means from July 16, 1992.

ANOVA

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	7.228	7.228	15.70***	3.88
Families	11	129.511	11.773	25.57***	1.83
Error	227	549.359	0.988		
Total	239	676.722			

ANOVA of Family Means of all progenies replicated twice.

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.560	0.560	7.16*	4.96
Families	10	15.432	1.543	19.70***	2.97
Error	10	0.783	0.078		
Total	21	16.776			

Components of Variance

Vfam = (1.543-0.078)/2 = 0.73
Ve = 0.08
Vp = 0.81

Broad-sense heritability for psyllid resistance = 0.903

Genetic Gain = i*s*h

i = 25% = 1.242
s = 0.078
h = 0.903

Gain (G) = 0.087

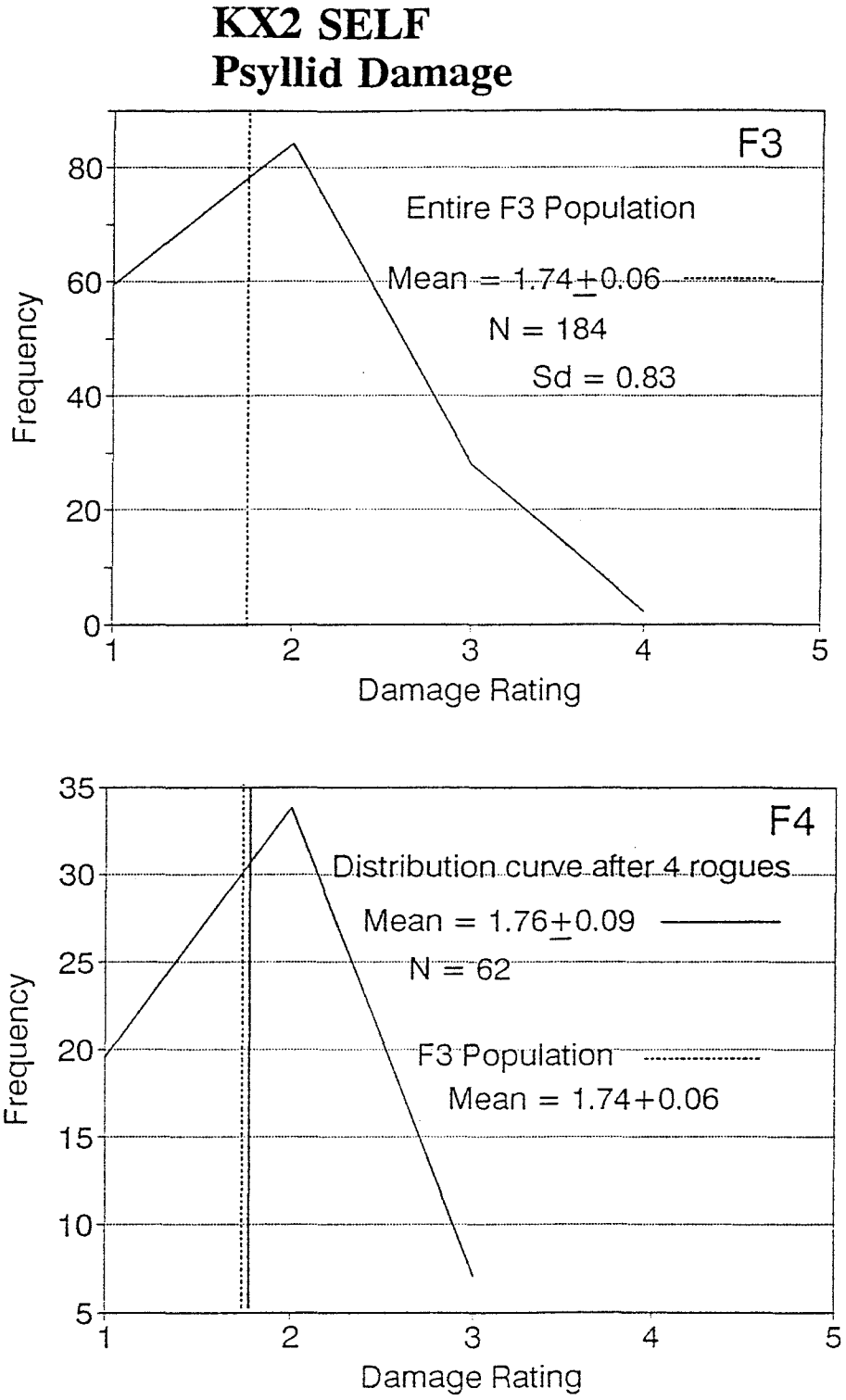

Figure 7.3. Variance distribution of F_3 and F_4 Leucaena KX2 self-pollinated (SELF) lines for psyllid damage taken May, 1994. Psyllid damage (1=no damage, 9= complete defoliation). Sd = standard deviation.

Table 7.9. Analysis of variance and estimates of heritability and genetic gain for vigor of KX2 SELF family means from Oct 18, 1992.

ANOVA

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	3.840	3.840	7.18**	3.88
Families	11	92.123	8.374	15.67***	1.83
Error	219	549.359	0.988		
Total	231	676.722			

ANOVA of Family Means of all progenies replicated twice.

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.057	0.057	0.76 NS	4.96
Families	10	9.303	0.930	12.33***	2.94
Error	10	0.754	0.075		
Total	21	10.115			

Components of Variance

Vfam = (0.930-0.075)/2 = 0.43
Ve = 0.075
Vp = 0.500

Broad-sense heritability for plant vigor = 0.860

Genetic Gain = i*s*h

i = 25% = 1.242
s= 0.075
h = 0.86

Gain (G) = 0.080

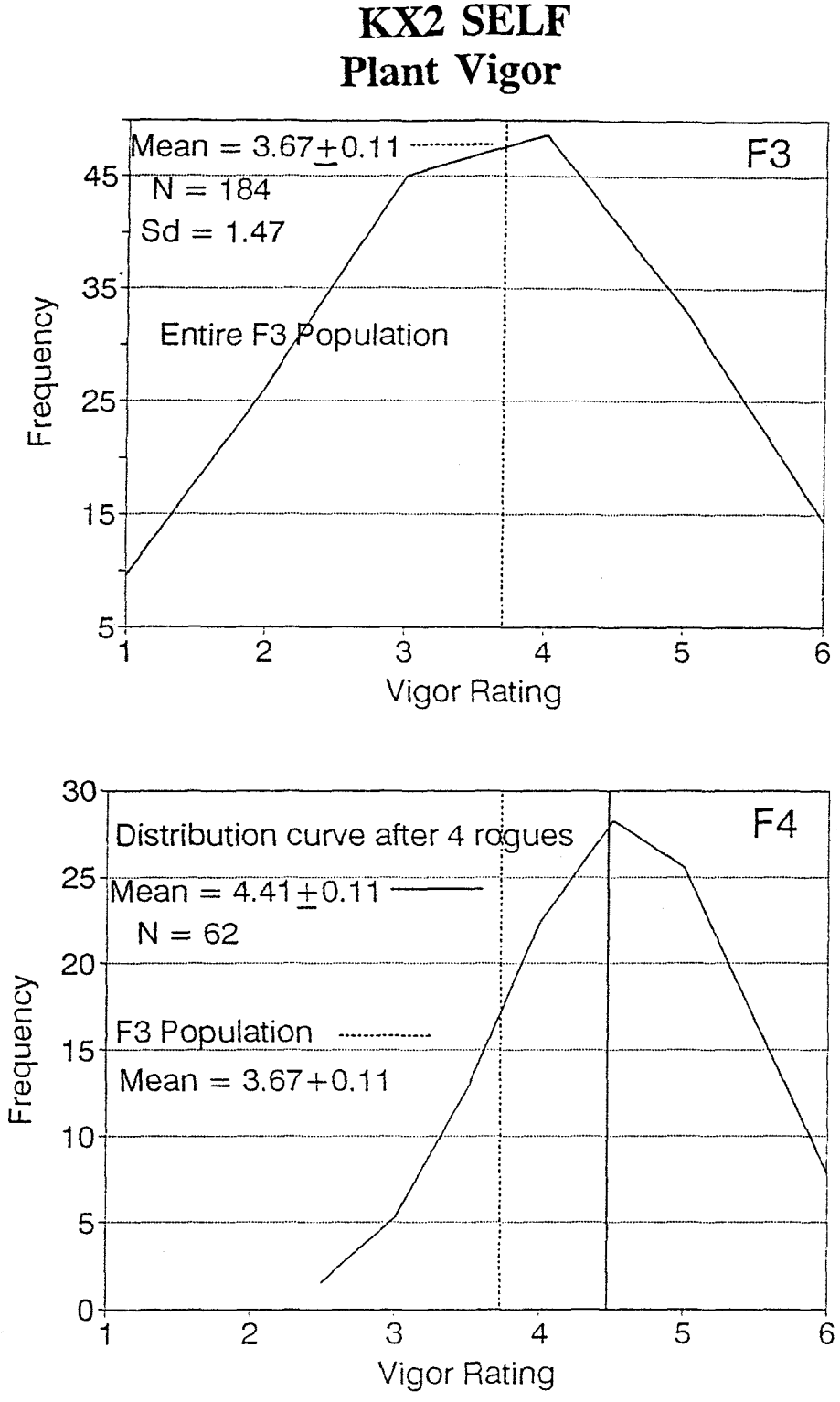

Figure 7.4. Variance distribution of F_3 and F_4 Leucaena KX2 self-pollinated (SELF) lines for vigor taken May, 1994. Vigor (1= low, 6= very high). Sd = standard deviation.

Table 7.10. Comparison between KX2 OP and KX2 SELF progenies for psyllid damage and vigor after 2 years and 4 rogues.

	Psyllid Damage			Vigor		
Family	SELF	OP	F_3	SELF	OP	F_3
1-3	2.8±0.10	2.8±0.16	3.0	3.7±0.18	4.2±0.73	3.0
2-4	2.0±0.35	1.0± .	2.5	3.9±0.47	3.5± .	3.0
3-26	1.6±0.24	2.4±0.39	3.5	4.8±0.14	5.2±0.26	6.0
5-3	1.5±0.22	1.1±0.07	1.0	4.0±0.42	3.7±0.37	5.0
5-20	2.2±0.17	1.7±0.18	1.0	4.8±0.44	4.8±0.26	5.5
5-36	1.3±0.25	1.1±0.11	1.0	4.8±0.75	5.1±0.28	6.0
6-2	3.0± .	1.0±0.00	1.0	3.0± .	3.5±0.00	3.5
6-9	1.0±0.00	2.0±0.00	1.0	4.7±0.25	4.3±0.75	4.5
6-15	1.3±0.14	1.2±0.09	1.0	4.5±0.17	4.8±0.29	5.0
6-34	1.7±0.33	2.1±0.27	2.0	4.7±0.16	3.9±0.27	4.5
7-5	1.0±0.04	N/A	1.0	5.6±0.15	N/A	5.5
Mean	1.76	1.64	1.63	4.41	4.27	4.68
CV	23	48	55	14	23	22
1-5		1.8±0.75	2.0		4.1±0.75	3.0
2-9		1.0±0.00	1.0		5.5±0.33	4.0
3-3		1.5±0.29	2.0		3.6±0.75	3.0
3-5		1.3±0.25	3.0		4.9±0.31	3.5
3-6		1.8±0.23	1.5		4.2±0.42	5.5
3-9		1.8±0.14	3.0		3.9±0.40	4.5
3-32		1.3±0.11	1.0		5.7±0.14	5.5
3-36		1.0± .	2.5		5.0± .	4.0
4-7		1.1±0.14	1.0		4.5±0.38	5.5
4-13		2.0± .	3.0		5.5± .	5.0
5-9		1.7±0.31	1.0		5.5±0.20	3.0
6-20		1.1±0.10	1.0		4.6±0.43	4.5
6-28		1.0± .	1.0		3.5± .	5.5
6-38		1.6±0.28	1.0		3.9±0.40	6.0
7-8		1.6±0.33	1.0		5.2±0.33	4.5
7-13		1.0±0.00	1.0		5.5±0.32	5.0
Mean		1.41	1.63		4.72	4.50
CV		41	49		24	22
Total Mean	1.76	1.49	1.74	4.41	4.54	3.67
CV	23	38	48	14	28	41

the KX2 SELF families and highest for the F_3 population (Table 7.10).

KX3

Of 612 trees planted, 75 % or 428 trees were rogued (Table 7.11). In contrast, an average of 59 % of the three top lines were rogued. Mortality was 10 % in this trial (Table 7.11).

A significant difference ($P<0.01$) between families was detected for psyllid damage after two months (Table 7.12).

The best KX3 lines were from parent trees 91-13 and 91-12, and 91-6. Progenies from these lines had high psyllid resistance and good vigor over all observations. Broad-sense heritability for family means of psyllid resistance was 0.645 and the expected gain for this trait was 0.12 (Table 7.12).

Mean psyllid damage of all progenies at the conclusion of the trial was 2.91 (Figure 7.5) while average psyllid damage of the three best lines was 1.84.

Vigor was also highly significant ($P<0.01$) between families with the best psyllid resistant lines also expressing the best vigor (Table 7.13). Broad-sense heritability for vigor was 0.701 and the expected gain was 0.198 (Table 7.13)

Vigor ratings of all progeny was 4.19 (Figure 7.6) while for the top three lines it was 5.14.

Table 7.11. Number of KX3 SELF trees rogued over time in SET 92-4 at Waimanalo, Hawaii.

				Rogue Number					
Tree #	Start	Died	Sub-Total	1	2	3	4	Rogue Total	%
91-1	40	2	38	6	13	13	2	34	89
91-2	24	1	23	7	5	4	1	17	74
91-3	40	1	39	13	5	6	3	27	69
91-5	35	1	34	8	8	13	1	30	88
91-6	40	0	40	12	7	5	4	28	70
91-7	32	0	32	11	4	7	2	24	75
91-8	40	0	40	8	11	7	3	29	73
91-9	40	5	35	5	9	11	4	29	83
91-10	40	12	28	3	6	14	2	25	89
91-11	40	3	37	14	6	1	0	21	57
91-12	40	6	34	8	7	6	1	22	65
91-13	40	2	38	6	10	3	2	21	55
91-14	23	2	21	4	5	2	2	13	62
91-15	8	0	8	3	1	0	0	4	50
91-16	8	0	8	2	1	0	0	3	38
91-17	30	4	26	3	10	6	2	21	81
91-18	37	1	36	0	9	22	2	33	92
91-19	40	9	31	0	5	14	6	25	81
91-20	40	17	23	0	9	9	4	22	96
TOTALS									
19	637	66	571	110	133	144	41	428	75

Table 7.12. Analysis of variance and estimates of heritability and genetic gain for psyllid resistance of KX3 SELF family means from 16 July 1992.

ANOVA

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.073	0.073	0.20	3.88
Families	18	238.993	13.277	35.29***	1.63
Error	592	222.713	0.376		
Total	611	461.750			

ANOVA of Family Means of all progenies replicated twice.

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.002	0.002	0.00 NS	4.41
Families	18	12.597	0.700	4.64**	2.22
Error	18	2.714	0.151		
Total	37	15.312			

Components of Variance

Vf = (0.700-0.151)/2 = 0.275
Ve = 0.151
Vp = 0.430

Broad-sense heritability for psyllid resistance = 0.645

Genetic Gain = i*s*h

i = 25% = 1.242
s= 0.151
h = 0.645

Gain (G) = 0.12

KX3
Psyllid Damage

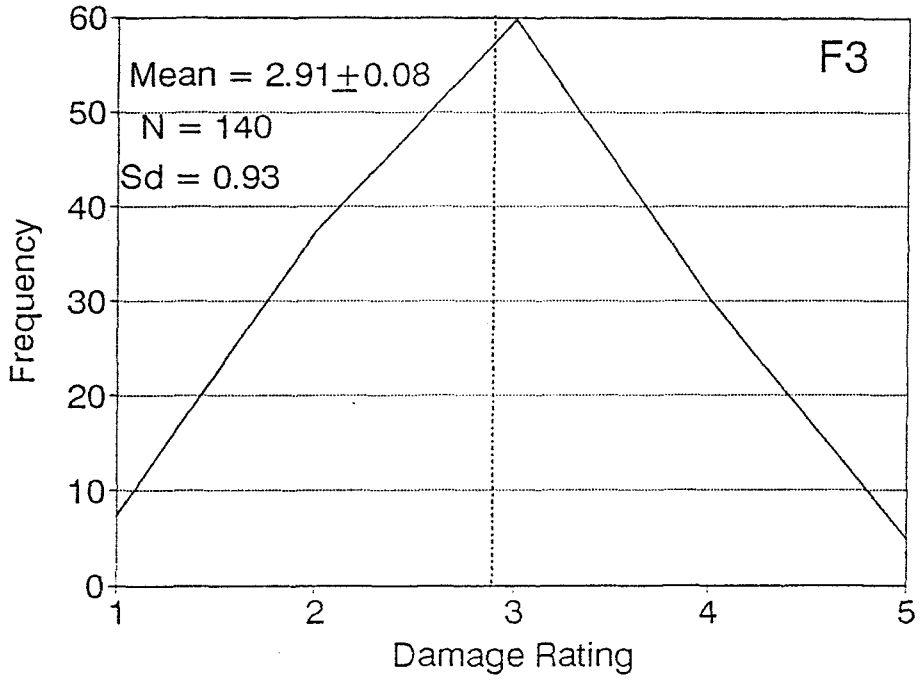

Figure 7.5. Variance distribution of Leucaena KX3 lines for psyllid damage taken May, 1994. Psyllid damage (1= no damage, 9= complete defoliation). Sd = standard deviation.

Table 7.13. Analysis of variance and estimates of heritability and genetic gain for vigor of KX3 SELF family means from 18 Oct 1992.

ANOVA

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	6.572	6.572	12.50**	3.88
Families	18	438.439	24.357	46.33***	1.63
Error	582	305.964	0.525		
Total	601	752.036			

ANOVA of Family Means of all progenies replicated twice.

Source	df	SS	MS	F	$F_{0.05}$
Rep	1	0.108	0.108	0.48 NS	4.41
Families	18	23.433	1.302	5.70**	2.22
Error	18	4.109	0.228		
Total	37	27.650			

Components of Variance

Vf = (1.302-0.228)/2 = 0.54
Ve = 0.228
Vp = 0.77

Broad-sense heritability for plant vigor = 0.701

Genetic Gain = i*s*h

i = 25% = 1.242
s= 0.228
h = 0.701

Gain (G) = 0.198

KX3
Plant Vigor

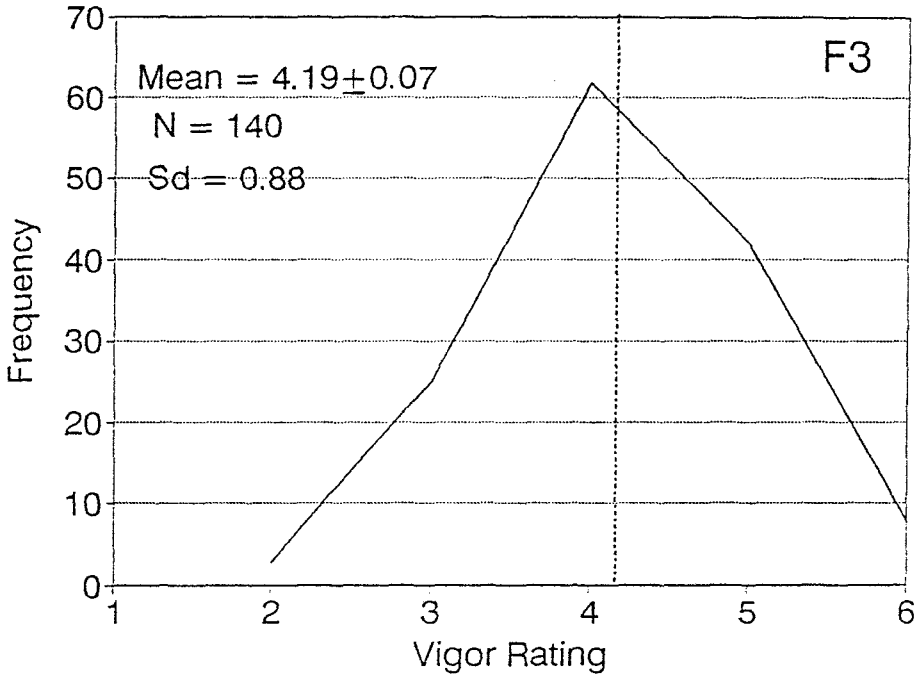

Figure 7.6. Variance distribution of Leucaena KX3 lines for vigor taken May, 1994. Vigor (1= low, 6= very high). Sd = standard deviation.

Psyllid damage and vigor were significantly ($P<0.001$) and negatively correlated at the conclusion of the trial ($r = -0.52$, $P<0.001$).

DISCUSSION

The initial selection of KX2 parent material from the F_3 population was affected due to seed availability. Selecting of the best parents from the initial population will give the best response (Matheson, 1990). In many instances in this experiment elite parent plants produced no seeds at the time of collection.

The number of progeny tested in this trial (20 in each replication) was similar to Cotterill and James (1984), low initial numbers for some families was due to poor germination. Roguing inferior trees during the experiment is an acceptable practice (Matheson and Raymond, 1984) and the overall percentage of trees rogued was consistent across KX2 OP, KX2 SELF and KX3 SELF lines. The percentage of trees selected for advancement (25 %) in this experiment is important for determining selection gains in future *Leucaena* breeding work.

Determining psyllid resistance of both KX2 parents and progeny was difficult in view of the already existing high resistance of the parents, and the erratic, and often times low psyllid epibiotics during the trial. Because of this a between-within analysis of variance for determining

heritability is probably a more reliable measure. The inherent high resistance of KX2 causes problems in accurately determining heritability by limiting the range in which psyllid damage can be scored. This in turn reduces the ability to accurately predict psyllid heritability through parent-progeny regression analysis. Psyllid damage ratings seldom exceeded a rating of 3 in both KX2 OP and SELF over two years, yet the rating scale is from 1 to 9. The difference between mean damage ratings of 1.49 and 1.74 is minimal, and probably not biologically significant. However, damage ratings for KX2 and KX3 in these trials were lower than average damage ratings of *L. leucocephala* entries (3.9) growing in an adjacent forage trial at the same time (Austin, Chapters 3 and 4). Damage h^2_b in both KX2 and KX3 of 37, 90, and 65 % is higher than Blada's (1980) report where h^2_b for *Aldeges laricis* resistance ranged from 8 to 15 %.

While components of vigor such as height, branch number, leaf area, leaf number, and forage biomass produced over time are all quantifiable, the large number of trees in the experiment required a method for determining vigor in a suitable time frame. The vigor scale encompassed the above traits and was easily detectable between families. The h^2_b of plant vigor of 26, 86, and 70 %, respectively indicates the greater homogeneity of the selfed lines as opposed to the open-pollinated progenies. Vigor estimates of KX2 OP in

this study were similar to Dean *et al.* (1986) who determined h^2_b of visual estimates for stem straightness, branch diameter, and branch angle to range from 0.14 to 0.49. Determining vigor had its own set of constraints in this study. The most noticeable was the difference between basal stem diameters of the F_3 parents and the F_4 progeny. The F_3 population was 5 years old in contrast to the progeny which were seedlings at the time. Vigor was also affected by the discrepancy in plant populations between the generations. Roguing was required in order to provide similar plant spacing for the F_4 which in effect negated the analysis of heritability at later stages of growth since heritability must be determined for the entire F_4 population.

Selection gains (G) should be determined using narrow-sense heritability (h^2_n). Gains reported from this study are based on h^2_b and must be interpreted with caution since the component of additive variance has not been separated from that of dominance and other variance interactions. The selection intensity coefficient of 1.242 was determined from Becker (1992) and was based on an average selection intensity of 25 % or 10 trees out of 40.

The significant negative correlation between psyllid damage and vigor for both KX2 and KX3 is an important finding. This means that breeders can select for both traits.

CONCLUSION

Psyllid damage in KX2 was lower than KX3, while forage vigor ratings were approximately the same. Genetic variances were higher for both psyllid resistance and vigor in the KX2 OP population than in the selfed KX2 and KX3 lines. There was a significant improvement for psyllid resistance for both KX2 and KX3 compared to *L. leucocephala* growing in adjacent plots. Excellent genetic advance for forage vigor of the KX2 F_4 progeny versus the average vigor of the F_3 population was observed. High vigor is most likely the result of reduced psyllid feeding pressure due to improved psyllid resistance. Significant correlation of low psyllid damage and high forage vigor assumes good genetic gains for both traits. The selection exerted on both KX2 and KX3 were similar, and can be used as a reference point for future experiments.

CHAPTER 8

TOTAL *LEUCAENA* BIOMASS PRODUCTION FROM TWO TRIALS PLANTED AT WAIMANALO, HAWAII

ABSTRACT

Identifying superior *Leucaena* genotypes for tropical environments is important for developing high wood yielding species. Height (HT), DBH, yields, and psyllid resistance were measured to quantify variation between *L. leucocephala* genotypes and to formulate the hypothesis that significant varaition exists within the species. Also correlations between seedling and mature tree height were tested to Two trials were planted at Waimanalo, Hawaii over an 11 year period. The first trial, SET 83-5 (SET is an acronym for State-Wide Energy Trial) ran from 1983 to 1987, while SET 91-3 ran from 1991 to 1994. *Leucaena leucocephala* (Lam.) De Wit K636 exhibited the best total DM biomass yield, height (HT) and diameter at breast height (DBH) during both trials. Predicted dry matter (DM) biomass yields ranged from 13 to 48 Mg ha^{-1} (3.25 to 12 Mg ha^{-1} yr^{-1}) in SET 83-5, and 1.1 to 113 Mg ha^{-1} (0.4 to 38 Mg ha^{-1} yr^{-1}) in SET 91-3. Significant variation occurred for HT ($P<0.001$) in each trial at year 1 and 2. HT ranged from 5.3 to 7.2 m in SET 83-5 after 4 years and 3.3 to 8.1 m in SET 91-3 after 3 years. DBH also differed ($P<0.001$) in both trials and ranged from 18 to 49

mm in SET 83-5 after 4 yr and 19 to 66 mm in SET 91-3 after 3 yr. Mortality was zero in SET 83-5 but was 21 % after three years in SET 91-3. K21 had the highest mortality with 63 %. Heights of three and six month old seedlings were correlated ($r=0.56$, $P<0.01$) with three year old trees in SET 91-3, suggesting that superior trees can be selected at an early growth stage in the field.

Reduced growth in SET 83-5 can be attributed to heavy psyllid damage after the first year. Psyllid damage of SET 83-5 was great owing to the fact that there were no biological controls at the time of infestation. K584, K636 and K638 had the lowest damage levels ($P<0.001$) during that trial. The best genotype emerging from both trials was K636, which is used extensively at the University of Hawaii as a parent in the *Leucaena* hybrid breeding program.

INTRODUCTION

Leucaena is an important wood producing tree in tropical environments. Native to Mexico and Central America, *Leucaena* spp. range from sea level to approximately 2000 m elevation (Brewbaker, 1987). There are approximately 16 to 17 species recognized in the genus *Leucaena* (Hughes, 1993). Of these, the predominately grown species is *L. leucocephala* (Lam.) de Wit which is cultivated on an estimated 2 million ha pantropically (Sorensson and Brewbaker, 1994). Four years appears to be a suitable

rotation age for *Leucaena* when planted in densities higher than 1000 ha^{-1} (NFTA, 1985). Peak rotation age based on mean annual increment (MAI) at Waimanalo for *L. diversifolia* x *L. leucocephala* KX3 planted in mixed and pure stands at 6600 plants ha^{-1} was 2 to 4 years (Austin, Chapter 6).

Wood production from giant leucaena *L. leucocephala* at Waimanalo, HI averaged 30 m^3 ha^{-1} yr^{-1} from 1982 to 1987 (Wheeler *et al.*, 1987). Dudley, (1990) reported yields of *L. leucocephala* K636 planted at 10,000 trees ha^{-1} at 20 Mg ha^{-1} (10 Mg ha^{-1} yr^{-1}) at Mt. View, Hawaii. Van den Beldt (1983) reported biomass yields of *L. leucocephala* at 10,000 stems ha^{-1} ranged from 37.3 to 96.5 Mg ha^{-1} over 3 years on 4 different islands (12 to 32 Mg ha^{-1} yr^{-1}). He reported a range of MAI for total biomass of 4 year old *L. leucocephala* trees at Waimanalo to be 15 to 19 Mg ha^{-1} yr^{-1}. Yields of between 30 to 50 m^3 ha^{-1} yr^{-1} have been reported in other tropical countries (NAS, 1980; NRC, 1984). Khot *et al.*, (1991) reported yields of *L. leucocephala* K8 in India at 78.8 Mg ha^{-1} (26.2 Mg ha^{-1} yr^{-1}) after 3 years spaced at 13,300 trees ha^{-1}. Height and DBH in his study were 7.8 m and 7 cm.

The NRC (1984a) states that heights of up to 18 m in 4 to 8 years can be realized under favorable conditions. After 24 months *L. leucocephala* K636 planted at 10,000 trees ha^{-1} realized an average height of 5.75 m and DBH of 3.68 cm over 6 different experimental sites (Dudley, 1990). Heights ranging from 7.4 to 10.3 m and DBH from 5 to 7 cm were

reported for *L. leucocephala* over 4 sites (Van den Beldt, 1983).

Sun (1992) tested 36 different giant *L. leucocephala* ssp. *glabrata* accessions for isozymic polymorphism. Of these, 15 were genotypes used in this study, the exception being K21 which was identified as 'common' leucocephala (*L. leucocephala* ssp. *leucocephala*). Seventy-five percent of the giant leucaenas showed polymorphisms at 4 of the 6 isozymes tested: Aconitase (ACO), Isocitrate dehydrogenase (IDH), Phosphoglucose isomerase (PGI) and Phosphoglucomutase (PGM) suggesting phenotypic variation within the species (Sun, 1992).

Psyllids (*Heteropsylla cubana* Crawford) invaded the Hawaiian islands in 1984 (Nakahara and Lai, 1984) and caused considerable damage to susceptible *Leucaena* species. The most commonly grown species *L. leucocephala* is highly susceptible to psyllid damage (Brewbaker *et al.*, 1989) but there are various levels of tolerance within the species (Wheeler, 1988). Psyllid populations increase dramatically when newly arrived to areas devoid of natural controls (Nakahara and Funasaki, 1986), but then equilibrate after approximately two years (Van den Beldt and Napompeth, 1992). Psyllids prefer actively growing leaf tips of the plant (Beardsley, 1986) and periodically cause damage to hedge-row managed *Leucaena* at Waimanalo (Austin, Chapters 3 and 4). However, when *Leucaena* is managed for wood production,

psyllid damage normally is marginal to trees after one year (Van den Beldt and Napompeth, 1992).

The objective of these experiments was to identify superior genotypes for lowland, tropical environments. Superiority was based on the best height and diameter and biomass yields. Significant variation of these measurements will substantiate earlier observations of the isozymic diversity of the species *L. leucocephala* ssp. glabrata. This research will enable the breeding program to recommend varieties useful for wood production systems in Hawaii and to use those varieties for interspecific hybridization with other species.

MATERIALS AND METHODS

The Waimanalo research station is located at 21^0 20′ N, 158^0 20′ W with a mean elevation of 20 m above sea level. Precipitation ranges from 1000 to 1800 mm yr^{-1}, mean annual temperature is 24.6^0C. Soils are colluvial underlaid by coral with a pH of approximately 6.0. Complete weather information for this site is available in Appendix A.

Two separate experiments SET 83-5 and SET 91-3 were conducted at Waimanalo during an eleven-year period for determining total biomass production for lowland sites in Hawaii. The first experiment (SET 83-5) was planted 19 April 1983. Full details as to genotypes and climate of SET 83-5 were reported by Wheeler *et al.* (1987). The trial

included 45 *L. leucocephala* genotypes planted in an augmented RCB, of which 40 genotypes were replicated three times with the remaining five genotypes augmented in single reps. Fourteen trees plot^{-1} were planted in double rows 1 m apart and spaced 1 m intrarow, plots were also spaced 1 m apart thus representing 10,000 plants ha^{-1}. Height and DBH were measured on the interior 10 trees in each plot at 12, 24 and 48 months after transplanting. No irrigation was applied during this trial and rainfall averaged 850 mm yr^{-1} over the 4 year period. Psyllid damage was rated in SET 83-5 using an empirical scale of 1-5 (1= no damage, 5= complete defoliation) in 1985. The heavy psyllid population in 1985 defoliated this trial heavily in 1985. A non-normal rating scale for psyllid damage required log-transformation. Transformed data were not normally distributed and data were tested non-parametrically by ranking the data in Proc NPAR1WAY (SAS, 1986).

SET 91-3, included the best 16 *Leucaena* varieties from SET 83-5 along with 8 untested genotypes (Table 8.1). Seeds were scarified in boiling water for 20 s, then drained and soaked for 30 m in tap water. Seeds were inoculated with 'C' type rhizobium (NIFTAL, Maui, HI) and planted 15 July 1991 into dibble tubes containing common planting medium (Sunshine Mix, Brewer Chemical, Honolulu, HI). Seedlings were watered 3 times daily for 2 months, and hardened outside for 2 wk prior to transplanting on 24 Sept. 1991.

Table 8.1. *Leucaena* genotypes planted 24 September 1991, in SET 91-3 at Waimanalo, Hawaii.

K number†	Genotype	Origin
K21	*L. leucocephala*	Philippines
K29	*L. leucocephala*	Honduras
K67	*L. leucocephala*	Salvador
K156	*L. diversifolia*	Mexico
K217	*L. leucocephala*	Salvador
K397	*L. leucocephala*	Mexico
K417	*L. leucocephala*	Salvador
K418	*L. leucocephala*	Salvador
K419	*L. leucocephala*	Salvador
K565	*L. leucocephala*	Mexico
K584	*L. leucocephala*	Mexico
K608	*L. leucocephala*	Mexico
K633	*L. leucocephala*	Mexico
K636	*L. leucocephala*	Mexico
K638	*L. leucocephala*	Mexico
K665	*L. leucocephala*	Mexico
K678	*L. leucocephala*	Thailand
K784	*L. diversifolia*	Mexico
K902	*L. macrophylla*	Mexico
K925	*L. shannonii*	Guatemala
K952	*L. lanceolata*	Mexico
KX1	*L. pallida* x *L. diversifolia*	
KX2	*L. pallida* x *L. leucocephala*	
KX3	*L. diversifolia* x *L. leucocephala*	

†K numbers are part of the University of Hawaii breeding designation and stands for Koa haole.

Seedlings were transplanted into a Isohyperthermic Vertic Haplustol that had been cultivated into a fine seed bed which previously was in maize production. Fourteen plants plot^{-1} were planted in a 1 m x 1 m spacing exactly as SET 83-5. Irrigation was used one time to establish the plants. Early weed control was done by spraying Roundup (Monsanto, Co.) at 2 % solution on 2 Nov. 1991.

The experimental design for SET 91-3 was a randomized complete block with 3 replicates and 24 selections. Eight measurements were taken over a three year period. Ten trees from within each plot were measured for height at 3, 6, 9, 12, 15, 18, 24 and 36 months after transplanting. Diameter measurement at breast height (DBH) were recorded at 9, 12, 15, 18, 24 and 36 months.

Data from the two trials, SET 83-5 and SET 91-3, were entered into a combined analysis representing the 16 genotypes common to both trials. If the interaction of genotype by measurement date was significant then the data were analyzed separately by date. Because crucial data for year 4 in SET 83-5 was missing, only years one and two were analyzed. In SET 91-3 height and diameter were analyzed separately by year with only year 3 statistically presented since ultimate growth was of main concern. Heterogeneous variance distribution for tree height and diameter in both experiments required a log transformation since error variance of measurements increased with increasing height

and diameter of the plant. Both parameters were analyzed using Proc GLM in SAS (1986). Mean separations were performed by Duncan-Waller.

Total above-ground biomass yields were predicted for trees at the conclusion of each experiment based on height and DBH using an allometric equation developed for *L. diversifolia* x *L. leucocephala* KX3. The allometric equation used for predicting total biomass was DM Biomass = $0.0135*DBH^{1.85}*HT^{1.50}$ (Austin, Chapter 6). Since height and diameter measurements of all trees in SET 83-5 were missing from year 4 total DM biomass was predicted using the diameter and height mean (Wheeler, 1987) from each genotype then multiplying by 10,000 trees ha^{-1} since mortality was zero. No further statistical analyses were performed for total DM biomass in this trial. Total DM biomass in SET 91-3 was predicted by applying the same allometric equation to individual trees in each plot, summing and then multiplying by the number of existing trees ha^{-1}. This in effect adjusts for mortality and provides more accuracy in predicting total DM biomass. Mean separations were performed on the transformed data using Duncan-Waller. Data were transformed back to their original values to facilitate understanding.

RESULTS

Mortality

There was no mortality in SET 83-5 after four years. Mortality in SET 91-3 was 21 % after three years and varied ($P<0.01$) between genotypes ranging from 0 for K217 and K418 to 63 % for K21 (Table 8.2). Total mortality increased yearly starting with 3 % in year 1, 8 % in year 2 and culminating with 13 % in year 3 (Table 8.2).

Biomass yields

Total biomass calculated for SET 83-5 ranged from 13 to 48 MG ha^{-1} after four years (3.25 to 12 Mg ha^{-1} yr^{-1}). The best yielding genotypes were K636 and K584 producing over 40 Mg ha^{-1}. A gap of greater than 10 Mg ha^{-1} was detected between these two selections and the remaining genotypes (Table 8.3).

Total biomass yields differed significantly ($P<0.001$) after three years and ranged from 1.1 to 113.4 Mg ha^{-1} (0.4 to 37.8 Mg ha^{-1} yr^{-1}) (Table 8.3). The *L. leucocephala* species in this trial exhibited a wide range in biomass yields, both the highest and lowest yields were attributed to this species. The highest yielding genotypes were K636, KX3, K397 and K584 producing an estimated 113.4, 91.6, 79.9 and 78.8 Mg ha^{-1}, respectively after three years (37.8, 30.5, 26.6 and 26.3 Mg ha^{-1} yr^{-1}, respectively).

Table 8.2. Mortality of *Leucaena* varieties after 1, 2 and 3 years at Waimanalo, Hawaii.

	Mortality (out of an initial 30 trees)			
Selection	Year 1	Year 2	Year 3	Total
K21	1	6	12	19
K29	0	3	1	4
K67	2	0	2	4
K156	3	0	12	15
K217	0	0	0	0
K397	2	0	0	2
K417	0	2	3	5
K418	0	0	0	0
K419	0	2	0	2
K565	0	2	0	2
K584	0	0	1	1
K608	1	0	0	1
K633	1	1	3	5
K636	0	6	0	6
K638	0	2	1	1
K665	3	12	2	17
K678	2	0	3	5
K784	0	1	5	6
K902	1	2	5	8
K925	0	1	3	4
K952	2	3	8	13
KX1	0	3	8	11
KX2	1	4	6	11
KX3	1	3	6	10
Total	20/720	53/700	81/647	154/720
Mortality %	3	8	13	21

Table 8.3. Total biomass yields (Mg ha^{-1}) and MAI (Mg ha^{-1} yr^{-1}) with LSD's of 24 *Leucaena* genotypes in SET 91-3 and 16 *Leucaena* genotypes in SET 83-5.

	-----SET 91-3-----		-----SET 83-5-----	
Genotype	Total Biomass†	MAI‡	Total Biomass	MAI
K636	113.4	37.8	48.1	12.0
KX3	91.6	30.5		
K397	79.9	26.6	18.6	4.6
K584	78.8	26.2	43.9	10.9
K565	71.6	23.9	17.7	4.4
K608	68.2	22.7	15.3	3.8
K902	65.1	21.7		
K784	58.4	19.5		
KX2	50.6	16.9		
K638	44.1	14.7	14.2	3.6
K419	36.8	12.3	18.2	4.6
KX1	35.8	11.9		
K925	28.8	9.6		
K678	26.2	8.7	17.7	4.4
K67	26.1	8.7	18.7	4.7
K418	24.4	8.1	14.8	3.7
K29	21.8	7.3	16.5	4.1
K633	17.8	5.9	15.2	3.8
K156	17.5	5.8		
K952	17.1	5.7		
K665	15.8	5.3	16.9	4.2
K217	15.8	5.3	16.4	4.1
K417	12.1	4.0	12.9	3.2
K21	1.1	0.4	29.1	7.3
Average§	42.5	14.2	20.9	5.2
$LSD_{0.05}$¶	34.7	11.6		

†Mean of total biomass leaves, twigs and stems/ha.
‡Mean of MAI. MAI = mean annual increment which is total biomass/3 years.
§Average total DM biomass of all trees in each trial.
¶LSD least significant differences. LSD not performed on SET 83-5 since 4 year data of the entire set was not available and therefore biomass and MAI should be cautiously interpreted.

The lowest yielding genotypes, K21, K417, K217 and K665 produced less than 16 Mg ha^{-1} (5.3 Mg ha^{-1} yr^{-1}). *Leucaena diversifolia* K784 produced significantly greater ($P<0.001$) biomass than K156 producing 58.4 Mg ha^{-1} (19.5 Mg ha^{-1} yr^{-1}) as compared to 17.5 MG ha^{-1} (5.8 Mg ha^{-1} yr^{-1}), respectively. *Leucaena macrophylla* K902 produced 65.2 Mg ha^{-1} (21.7 Mg ha^{-1} yr^{-1}), which was higher ($P<0.05$) than both *L. shannonii* K925 and *L. lanceolata* ssp. sousae K952 which produced 28.8 and 17.1 Mg ha^{-1} (9.6 and 5.7 Mg ha^{-1} yr^{-1}), respectively (Table 8.3).

Height and diameter

The entire data set for the four year measurement in SET 83-5 was not available and only means are reported. Plant height after four years in SET 83-5 ranged from 5.4 to 7.2 m for K67 and K636, respectively (Figure 8.1). Diameter at breast height ranged from 30 to 49 mm for K417 and K636, respectively (Figure 8.2).

Heights in SET 91-3 differed ($P<0.001$) across treatments after three years (Table 8.4) and ranged from 3.2 to 8.2 m (Figures 8.3-8.5). *Leucaena leucocephala* K636 obtained the greatest height with 8.2 m after three years and was closely followed by KX3 with a mean height of 8.0 m (Figure 8.5). Eight genotypes averaged under six meters in height after three years these were in descending order

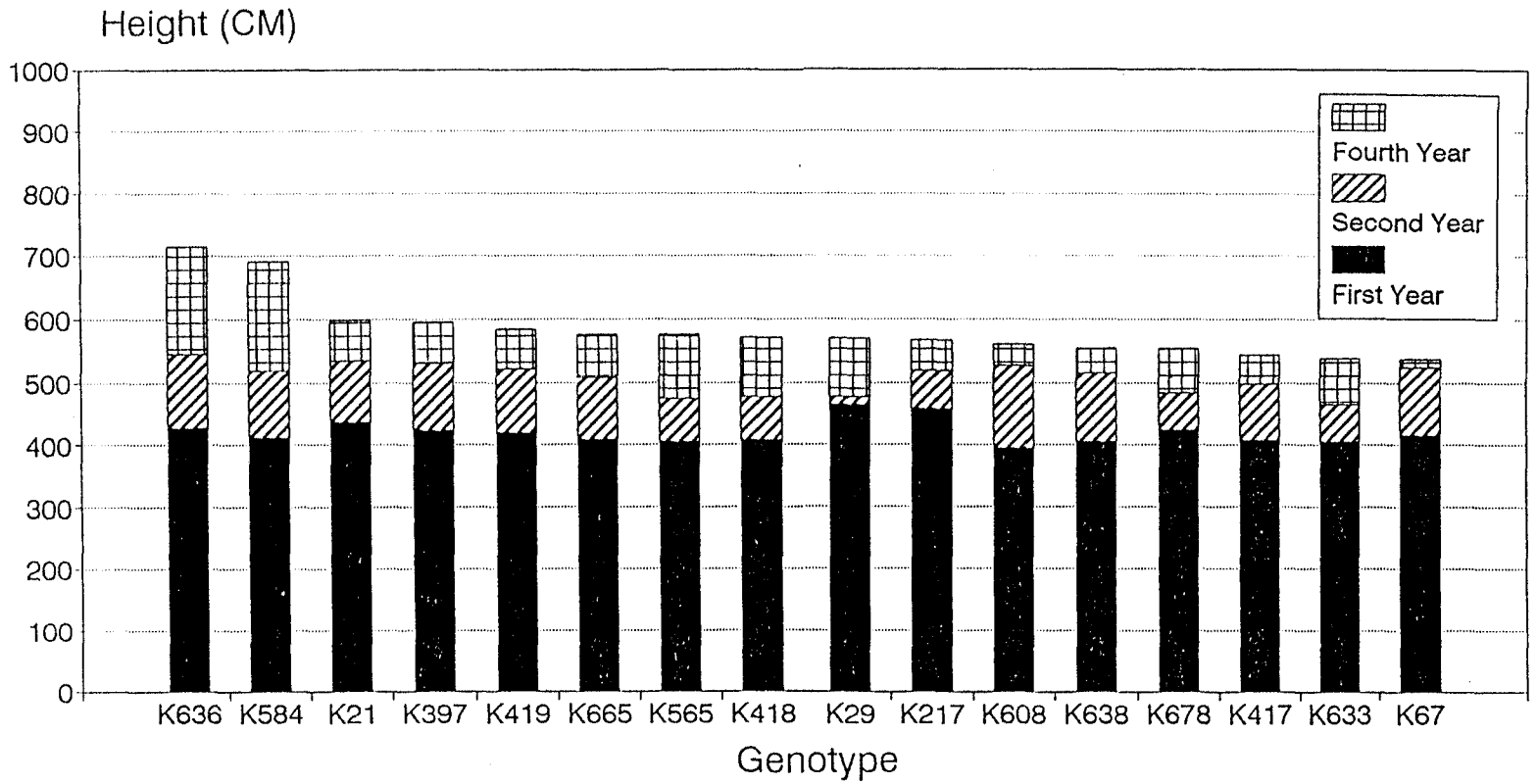

Figure 8.1. Height (cm) of 16 *L. leucocephala* genotypes after 4 years in SET 83-5 at Waimanalo, Hawaii grown from 1983-1987.

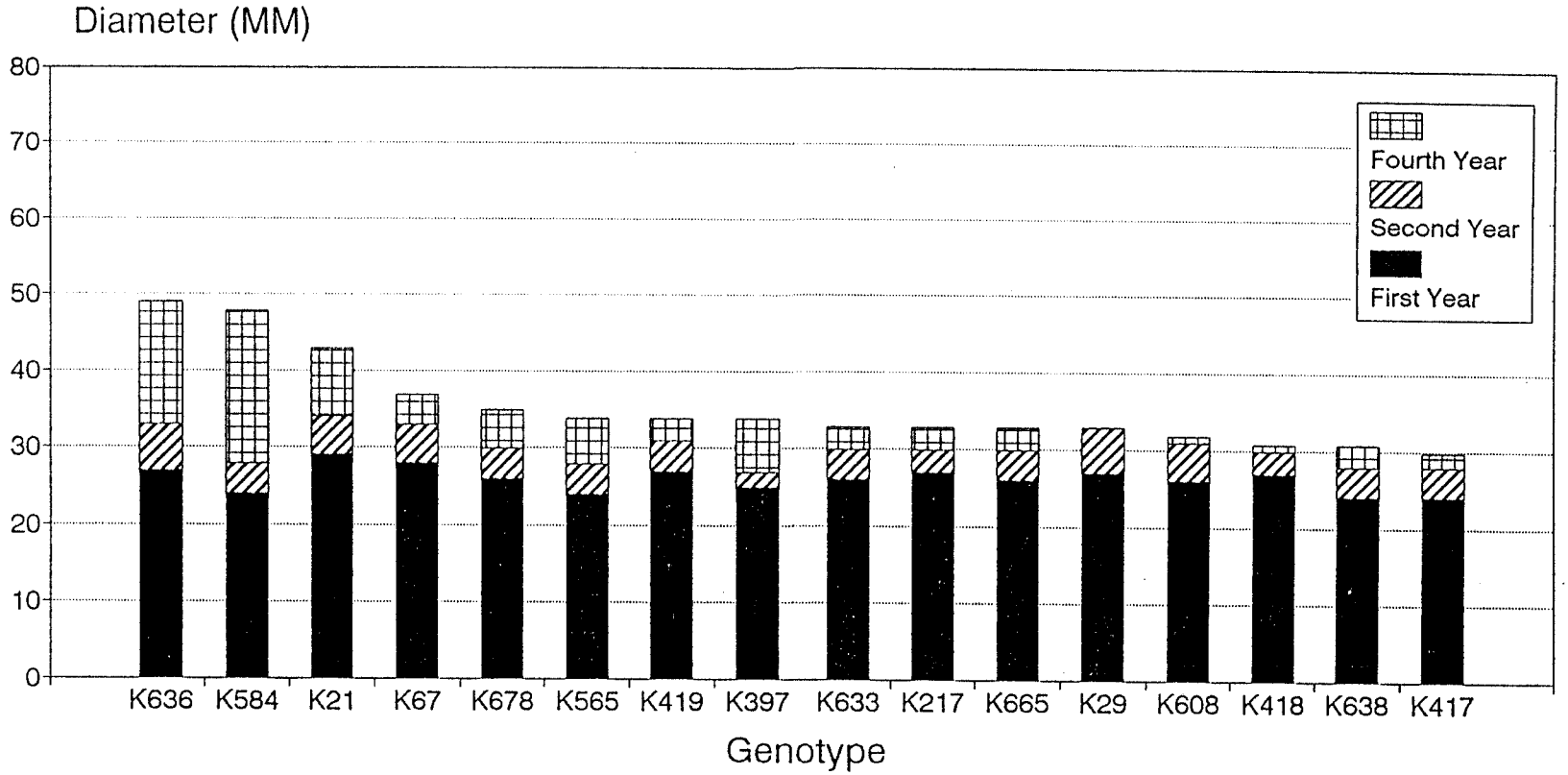

Figure 8.2. Diameter at breast height (mm) of 16 *L. leucocephala* genotypes after 4 years in SET 83-5 at Waimanalo, Hawaii grown from 1983-1987.

K418, K156, K67, K678, K665, K217, K417 and K21 (Figure 8.5).

Diameter at breast height differed ($P<0.001$) and ranged from 19 mm to 66 mm (Table 8.4). *L. leucocephala* K636 and the F_2 hybrid KX3 had the highest DBH ($P<0.05$) with 66 and 63 mm. The lowest DBH ranged from 31 mm for K678 to 19 mm for K21. This represents 1/2 to 1/3 the DBH of K636 (Figure 8.6).

Height and diameter for the 16 genotypes planted in both trials had significantly different ($P>0.001$) location, and genotype by location interaction at years one and two (Table 8.5), therefore each trial was analyzed separately. In SET 83-5 plant height did not differ across all genotypes in year 1 but there were genotypic differences ($P<0.001$) in DBH (Table 8.6). In year two, both height and DBH differed ($P<0.01$) between the 40 replicated genotypes (Table 8.6).

Tree heights at 3, 6, 9 and 12 months in SET 91-3 were correlated with heights after three years. Heights at three and six months were moderately correlated ($r=0.56$, $P<0.01$) to three year old trees. Correlations improved at 9 months ($r=0.77$, $P<0.001$) and still further at 12 months ($r=0.84$, $P<0.001$) indicating that early height in the field is correlated to later height of *Leucaena*.

Table 8.4. ANOVA of three year total biomass yields of 24 *Leucaena* genotypes grown in SET 91-3 at Waimanalo, Hawaii. Type III sum of squares.

Source	df	SS (10^{-6})	MS	F
BLK	2	645.97	322.99	0.72 NS
GENOTYPE	23	59963.59	2607.11	5.83 ***
Error	46	20556.64	446.88	
Total	71	81166.20		

*** significant to P<0.001 probability level, and NS = not significant.

Table 8.5. ANOVA of three year height, and DBH of 24 *Leucaena* genotypes grown in SET 91-3 at Waimanalo, Hawaii. Type III sum of squares

Log-Transformed Data

MS Values

Source	df	Ht	F	DBH	F
BLK	2	0.62	6.91 **	0.73	3.03 *
GENOTYPE	23	0.89	10.02 ***	2.70	11.14 ***
Error	540	0.089		0.24	
Total	565				

Non-Transformed Data

MS Values

Source	df	Ht	F	DBH	F
BLK	2	133744	5.02 **	401	0.98 NS
GENOTYPE	23	248854	9.33 ***	3702	9.03 ***
Error	540	26666		410	
Total	565				

*,**,*** significant to P< 0.05, 0.01 and 0.001 probability level, and NS = not significant.

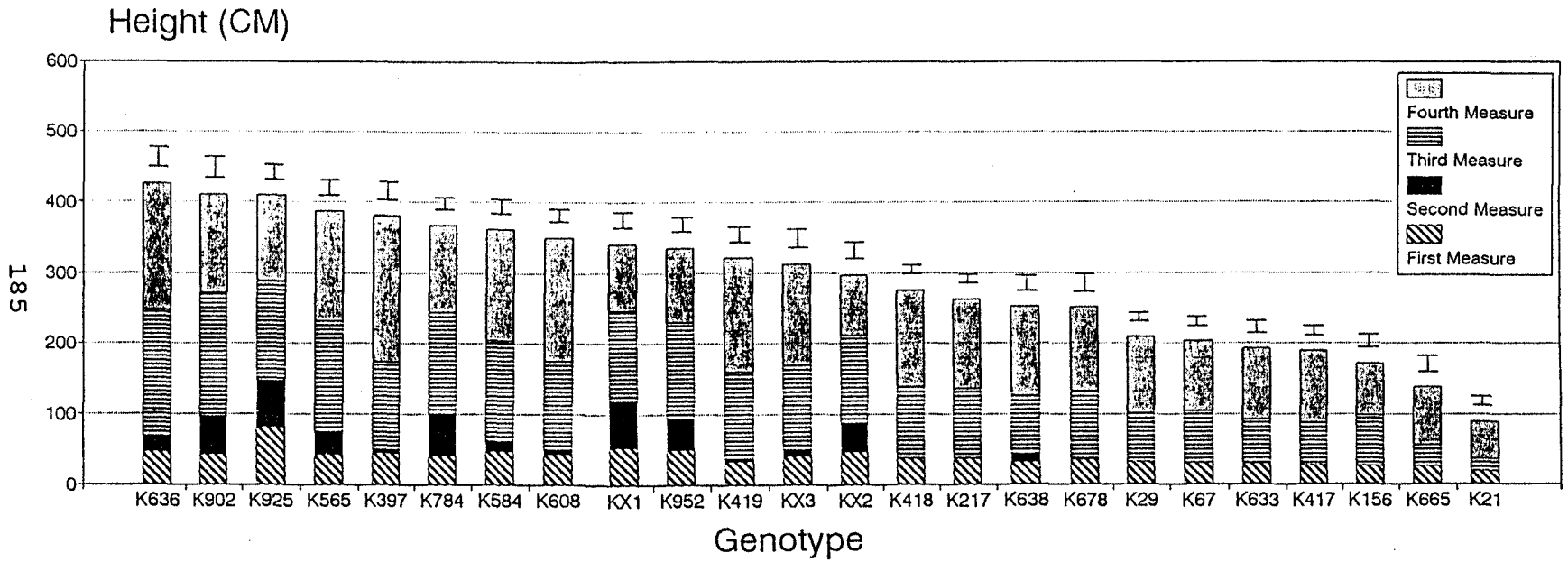

Figure 8.3. Height (cm) and standard errors of 24 *Leucaena* genotypes after 1 year in SET 91-3 at Waimanalo, Hawaii grown from 1991-1994. *L. leucocephala* = K21, K29, K67, K217, K397, K417, K418, K419, K565, K584, K608, K633, K636, K665, K678; *L. diversifolia* = K156, K784; *L. macrophylla* = K902; *L. shannonii* = K925; *L. lanceolata* = K952; *Leucaena* Hybrids = KX1, KX2 and KX3.

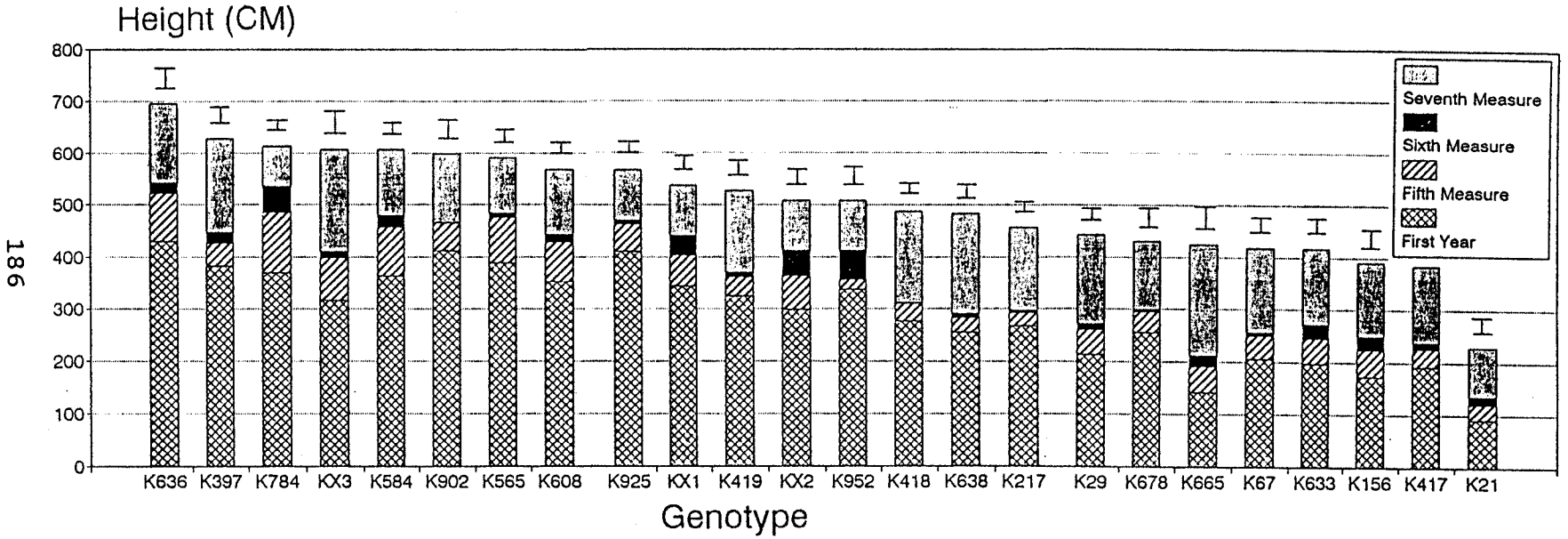

Figure 8.4. Height (cm) and standard errors of 24 *Leucaena* genotypes after 2 years in SET 91-3 at Waimanalo, Hawaii grown from 1991-1994. *L. leucocephala* = K21, K29, K67, K217, K397, K417, K418, K419, K565, K584, K608, K633, K636, K665, K678; *L. diversifolia* = K156, K784; *L. macrophylla* = K902; *L. shannonii* = K925; *L. lanceolata* = K952; *Leucaena* Hybrids = KX1, KX2 and KX3.

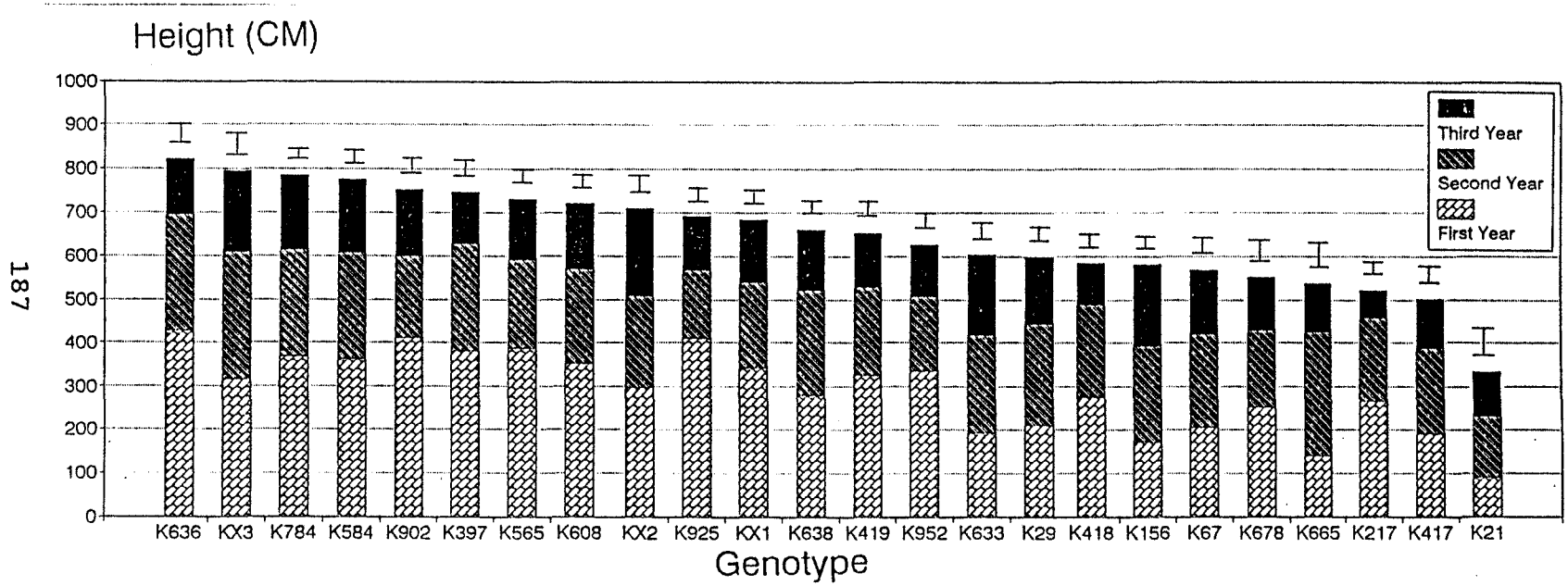

Figure 8.5. Height (cm) and standard errors of 24 *Leucaena* genotypes after 3 years in SET 91-3 at Waimanalo, Hawaii grown from 1991-1994. *L. leucocephala* = K21, K29, K67, K217, K397, K417, K418, K419, K565, K584, K608, K633, K636, K665, K678; *L. diversifolia* = K156, K784; *L. macrophylla* = K902; *L. shannonii* = K925; *L. lanceolata* = K952; *Leucaena* Hybrids = KX1, KX2 and KX3.

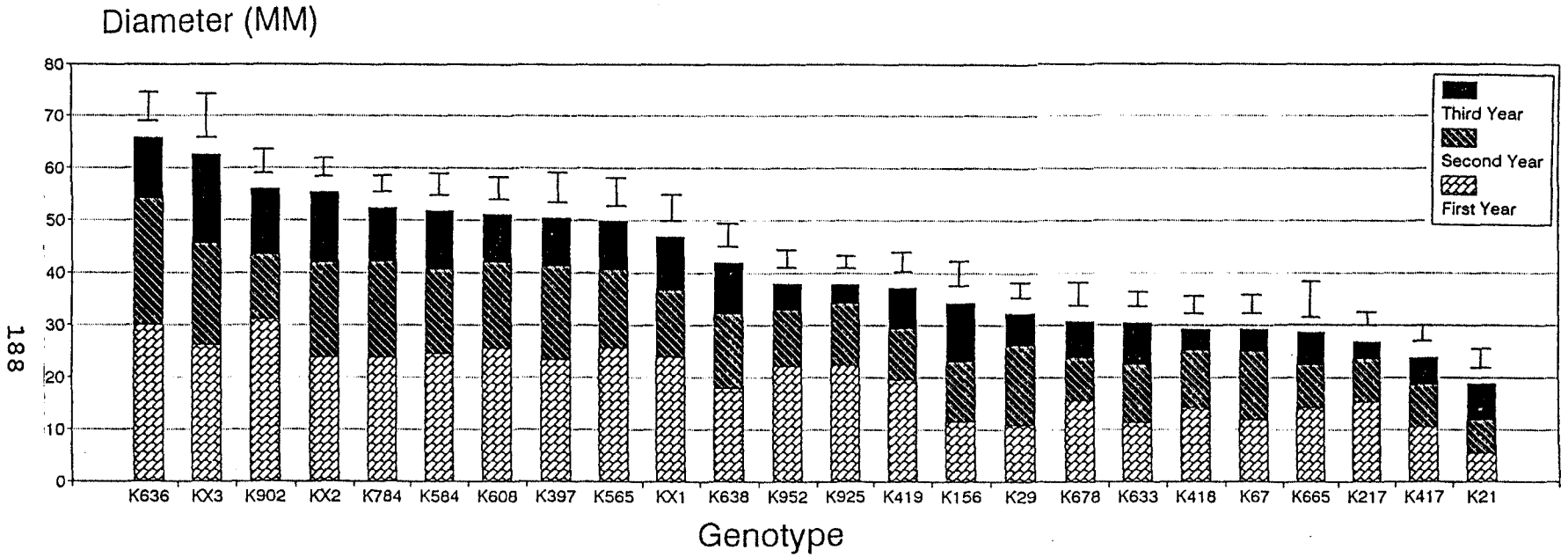

Figure 8.6. Diameter at breast height (mm) and standard errors of 24 *Leucaena* genotypes after 3 years in SET 91-3 at Waimanalo, Hawaii grown from 1991-1994. *L. leucocephala* = K21, K29, K67, K217, K397, K417, K418, K419, K565, K584, K608, K633, K636, K665, K678; *L. diversifolia* = K156, K784; *L. macrophylla* = K902; *L. shannonii* = K925; *L. lanceolata* = K952; *Leucaena* Hybrids = KX1, KX2 and KX3.

Table 8.6. ANOVA of one and two year log-transformed height and DBH of 16 *Leucaena* genotypes grown in SET 83-5 and SET 91-3 at Waimanalo, Hawaii. Type III sum of squares.

		Year 1		Year 2	
Source	df	Ht	DBH	Ht	DBH
LOC	1	6.79 **	16.24***	0.112 NS	0.026 NS
BLK(LOC)	4	0.16	0.64	0.075	0.192
GENOTYPE	15	0.26***	0.95***	0.109***	0.274***
GENxLOC	15	0.28***	1.07***	0.105***	0.308***
ERROR b	60	0.025	0.096	0.015	0.068
Total	95				

,* significant at P<0.01 and 0.001 probability level.

Table 8.7. ANOVA of height and DBH of 40 *Leucaena* genotypes grown in SET 83-5 at Waimanalo, Hawaii. Type III sum of squares.

Log-Transformed Data

MS Values

		Year 1		Year 2	
Source	df	Ht	DBH	Ht	DBH
BLK	2	0.009 NS	0.44***	0.109***	0.567***
GENOTYPE	39	0.202 NS	0.04***	0.027**	0.039***
Error	78	0.192	0.014	0.014	0.011
Total	119				

Non-Transformed Data

MS Values

		Year 1		Year 2	
Source	df	Ht	DBH	Ht	DBH
BLK	2	12122 *	266.7***	24949***	461.2***
GENOTYPE	39	4906 NS	23.8***	5388**	27.7***
Error	78	3235	7.3	2892	7.8
Total	119				

*, **, *** significant to P<0.05, P<0.01 and P<0.001 probability level, and NS = not significant.

Shorter HT increments during winter months (January to March) for all genotypes in SET 91-3 in years one and two were detected and are represented as the second and sixth measures on Figures 8.3 and 8.4. Average daily solar radiation levels during winter months are less than 50% of those measured in summer and are probably responsible for the lower relative growth rate since both rainfall and temperature were not limiting (Appendix A).

Psyllid damage

Only SET 83-5 was rated for psyllid damage. Mean psyllid damage for the entire trial at year two was 4.3 representing considerable leaf loss when based on a 1 to 5 scale. Psyllid damage differed between genotypes ($P<0.001$) at year 2 with K584 (2.9), K636 (3.5) and K638 (3.5) having the lowest damage ratings (Figure 8.7), with the remaining genotypes have similar damage ratings.

DISCUSSION

Differences in mortality between SET 83-5 and SET 91-3 is due to interspecific competition in SET 91-3 and psyllid preference for some varieties over others. Clear differences in psyllid tolerance among *L. leucocephala* genotypes in SET 83-5 and the fact that this trial was planted one year before psyllids suggest that all genotypes had equal opportunities as seedlings.

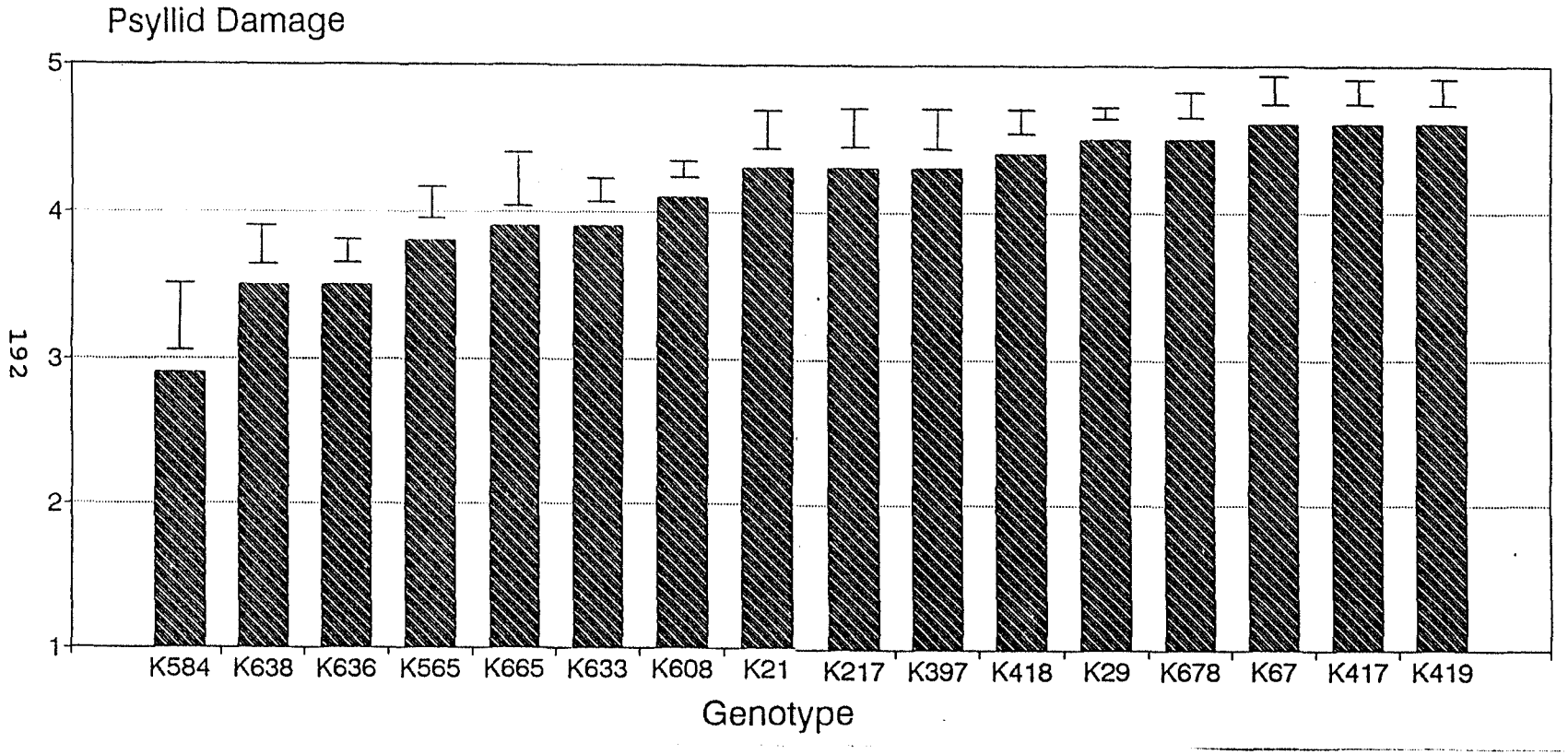

Figure 8.7. Psyllid damage (1=no damage, 5=complete defoliation) and standard errors of 16 *L. leucocephala* genotypes at year 2 in SET 83-5 at Waimanalo, Hawaii grown from 1983-1987.

Although there were statistical differences between genotypes in SET 83-5 at year two for height and DBH, not a single genotype dominated until year four when K636 and K584 emerged as the tallest trees.

Twenty-one percent mortality in SET 91-3 is not uncommon in tree stands of this density. Mortality of pure *Eucalyptus* stands at high densities ranged from 0 to 39 % in Hawaii (DeBell *et al.*, 1989; Whitesell *et al.*, 1988). The fact that mortality increased for the smallest trees i.e. K21, K156, and K665 in year 2 and 3 suggests that competition from larger trees caused this event probably as the result of psyllid damage on these more susceptible genotypes. In fact, K21 is no longer considered a giant *L. leucocephala* genotype (Sun, 1992), isozymic analysis considers this line to be 'common' leucaena.

Total biomass yields from both experiments should be interpreted with caution since the experimental design was not appropriate for determining biomass production. The double row planting arrangement does not consider intraspecific competition since both sides of the plot are surrounded by differebnt species or genotypes. Instead, these trials were intended to determine superior genotypes for lowland, tropical environments. However, the total biomass yields do serve as a guide to determining the best genotypes and are presented under this caveat. Total biomass yields from SET 83-5 were low due to psyllid

pressure and drought. Psyllids were first observed approximately one year after establishment and started damaging plants approximately six months later (18 months after transplanting). At the two-year mark, psyllids had no natural enemies to contend with and caused estimated leaf loss of up to 75 %. A comparison between SET 83-5 height at two and four years against height increment of SET 91-3 shows how growth was stunted. Below average rainfall (less than 1300 mm annum^{-1} was recorded all 4 years at Waimanalo during set 83-5. Rainfall averaged 522, 835, 1170 and 875 mm yr^{-1} from 1983 to 1986, respectively.

Total DM biomass yields of K636, KX3, K397 and K584 in SET 91-3 were higher than 25 Mg ha^{-1} yr^{-1} which is thought to be the upper level of tree biomass production in Hawaii (J. Fownes, personal communication), and higher than previously reported DM biomass yields of *Leucaena*. Van den Beldt (1983) reported MAI of wood yields of 15 to 19 Mg ha^{-1} yr^{-1} from 4 year old *L. leucocephala* trees in Waimanalo, and 12 to 32 Mg ha^{-1} yr^{-1} for three year old trees on four different islands. KX3 produced 22 Mg ha^{-1} yr^{-1} total DM biomass from an earlier forage biomass trial at Waimanalo (Austin, Chapter 2). Total DM yield of *L. diversifolia* K784 was over three times higher than K156. Based on this and earlier observations, K784 should be used for future KX3 combinations with the best *L. leucocephala* genotypes. The yields of *L. shannonii* K925 and *L. lanceolata* K952 were low

even though both are native to lowland environments (Brewbaker, 1987b). *Leucaena macrophylla* K902 yielded moderately well and is commonly found at higher elevations (Brewbaker, 1987b).

The significant interaction between location and genotype for height and diameter for the 16 *L. leucocephala* genotypes planted in both experiments is probably the result of interspecific competition in SET 91-3 in year one, as well as the degree of psyllid infestation (none in SET 83-5 in yr one) and varying psyllid resistance of the plants. Differences between trials concerning HT and DBH in year 2 are noticeable and are most likely due to a combination of severe defoliation from psyllids, and drought in SET 83-5. Rainfall was extremely low in 1983 with 522 mm and again in 1984 with 835 mm. Both represent a considerable deviation from the average rainfall of 1300 mm at Waimanalo.

Height differences at six months in SET 91-3 are due to several factors such as differences in carbon partitioning between species, and lower winter solar radiation levels and temperature at Waimanalo (Appendix A). The best height at six months were attributed to K902, K925, K784, KX1, KX2 and K952. Sorensson *et al.* (1994) reported differences in carbon partitioning between *Leucaena* species in an earlier trial at Waimanalo. In that study, *L. leucocephala* root biomass as a percentage of total DM after 84 days ranged from 35-39 % as compared to 26 % for *L. lanceolata* K393.

Lower winter solar radiation levels (less than 50 % of summer levels) may also be responsible for differences in height increment at 6 months as well as a 4 ^{0}C drop between summer and winter temperatures. Average height and diameter in SET 91-3 were somewhat smaller than earlier reports for *L. leucocephala* at Waimanalo. Low rainfall in 1993 may have contributed to the overall reduction in growth (Appendix A). Trees planted in a 1m x 1m spacing had heights of 5.0 m and DBH of 30 mm after two years (Dudley, 1990), 9 m height and 57 mm DBH after three years, and 9.9 m height and 67 mm after four years (Van den Beldt, 1983).

Early psyllid infestations cause considerable damage to susceptible *Leucaena* species (Nakahara and Funasaki, 1986) because natural controls either trail far behind psyllid (Funasaki *et al.*, 1989) introductions into new areas or cannot cross barriers such as large bodies of water. Apparently, a severe psyllid epibiotic lasted for approximately two years in Hawaii before psyllid populations leveled off. Unfortunately, trial SET 83-5 was a little over one year old at the time and bore the brunt of the psyllid invasion. Early psyllid population explosion and consequent damage to susceptible genotypes when newly introduced has occurred elsewhere. An example is the complete defoliation of *Leucaena* was Florida in 1983 (Othman and Prine, 1984). Infestation generally levels off after two years when psyllid population equilibrate (Van den Beldt

and Napompeth, 1992) and tolerant *L. leucocephala* genotypes usually outgrow infestation damage when managed for wood production (M.T. Austin, personal observation). Because psyllids go from being an acute pest in the first two years to an occasional pest thereafter, SET 91-3 had the benefit of shorter peak psyllid infestations.

CONCLUSIONS

Leucaena leucocephala K636 was the best genotype for height, diameter and total DM biomass in both trials. Other *L. leucocephala* genotypes that performed well in both trials were K584, K397 and K565. The F_2 hybrid KX3 and *L. diversifolia* K784 complete the top six genotypes from both trials. The good performance of KX3 (*L. diversifolia* K156 x *L. leucocephala* K636) may be improved by using K784 as one parent in place of K156. The large leaved *Leucaena* genotypes did not produce sufficient biomass to warrant further study with the possible exception of *L. macrophylla* K902. Leaf damage caused by psyllids decreased after the first two years of the initial infestation period. Large variation in HT, DBH, and biomass production was observed between the self-pollinating giant *L. leucocephala* genotypes a fact that supports variation within the species.

CHAPTER 9

A STERILIZATION PROCEDURE FOR MICROPROPAGATION OF FIELD GROWN *LEUCAENA* SPECIES

ABSTRACT

Sterilization procedures for micropropagating *Leucaena* spp. are often developed around material grown in the greenhouse (i.e., "soft" environments). Various sterilization techniques were investigated to develop a reliable system of sterilization for micropropagating superior field-tested genotypes of *Leucaena*, and to determine whether seasonal variation affects sterilization success. Explant material was taken from mature, regularly coppiced field-grown trees of triploid *L. esculenta* x *L. leucocephala* (TRIP) and arboreal *L. pallida* K804 (PALL) at Waimanalo, Hawaii. Two to three cm long explants containing a single bud were prepared in the laboratory and placed in 1/2 Murashige and Skoog (MS) gel in test tubes. The highest percentage of sterile explants (36 to 60 % pathogen-free) came from variations on a 3 second dip in 90 % EtOH, a 10 minute dip in 20 % v/v chlorox bleach, followed by a 10 minute dip in 0.1 % $HgCl_2$. Explants taken in summer (high light, low rainfall periods) were easier to sterilize than when taken in winter. Since shoot proliferation of *Leucaena* explants is reportedly low, reliable sterilization

represents a requisite strategy for developing large numbers of explants.

INTRODUCTION

Vegetative propagation of *Leucaena* spp. has shown varying success rates around the world and Hawaii (Dhawan and Bhojwani, 1985; Brewbaker, 1987d). Hu and Liu, (1981) reported up to 100 % root strike of softwood cuttings from 1 year old ortets under frequent misting in Taiwan, while Bristow (1983) reported high rooting efficiency of *L. leucocephala* cuttings (up to 100%) in a Welsh greenhouse after wilting the cuttings under lights for 60 minutes. However, neither of these methods has worked in Hawaii on various *Leucaena* species.

Micropropagation is seen as an alternative to cutting propagation for producing clonal material. Cloning would enable plant breeders to utilize self-incompatible tetraploid and diploid species such as *L. pallida* and *L. esculenta* for both fertile and sterile (triplod) hybrid seed production (Brewbaker, 1982). Clonal material can also be used for establishing homogeneous pure-line accessions for testing environmental effects by eliminating genotypic variation (Cotterill *et al.*, 1990; Farmer and Wilcox, 1968), or to develop clonal tree plantations since self-incompatible *Leucaena* species do not breed true (Brewbaker, 1982).

The majority of *Leucaena* micropropagation reported thus far has used explant material taken from greenhouse or laboratory grown plants (Dhawan and Bhojwani, 1985; Goyal *et al*. 1985). However, selection of superior individual plants requires mature, field-evaluated trees. Plants growing outdoors are subject to higher rates of infection than plants grown in greenhouse or laboratory conditions and are more susceptible to modifications in sterilization techniques and difficulties in rooting and shoot proliferation (Hartmann *et al*., 1990; Durzan, 1983). Contaminants are usually present on the plant surface, although they may be lodged inside difficult areas (Hartmann *et al*., 1990). Internal pathogens such as viruses and bacteria can inhibit growth or kill the plant outright (Hartmann *et al*., 1990).

Dhawan and Bhojwani (1985) reported 70 to 80 % sterile cultures from 9-month old *L. leucocephala* K8 plants after using a quick dip in ethanol (90 %), rinsing in 1 % cetavlon (CTAB) solution for 5 minutes, and 1 % chlorox for 15 minutes with appropriate rinses in sterile distilled water. Goyal *et al*. (1985) working on *L. leucocephala* K67 used a quick dip in EtOH (90 %), a 5 minute dip in 20 % (v/v) solution of sodium hypochlorite bleach, 3 rinses in distilled water, 5 minutes in 0.1 % mercuric chloride ($HgCl_2$) and another 3 rinses in distilled water but did not report percent contamination.

Optimum temperature for shoot proliferation should be between 27 and 30 degrees C (Dhawan and Bhojwani, 1985) with relative humidity (RH) of 50 to 60 % (Goyal *et al.*, 1985; Venketeswaran *et al.*, 1984). Light intensity and duration was recommended to be 60 micro Einsteins m^{-2} s^{-1} for approximately 16 to 18 hours d^{-1} by Goyal and Arya, (1984) and Goyal *et al.* (1985). Shoot initiation was detected within one week, and 2 to 3 shoots were evident after one month (Goyal *et al.*, 1985). Shoot proliferation of *L. leucocephala* was minimal in most studies, initially only one or two shoots are produced (Dhawan and Bhojwani, 1985; Goyal *et al.*, 1985). The best shoot production reported was with 3.0 mg l^{-1} BA (N-6 benzyladenine) and 0.05 mg l^{-1} NAA (napthaleneacetic acid) (Goyal *et al.*, 1985).

The objective of this study was to develop sterile micropropagules from plant material grown outdoors as opposed to material that has come from greenhouse environments in order to capture superior genotypes for developing seed orchards.

MATERIALS AND METHODS

The Waimanalo research station is located at 21^0 20′ N, 158^0 20′ W with a mean elevation of 20 m above sea level. Precipitation ranges from 1000 to 1800 mm $year^{-1}$, with the majority of rain falling in the winter months. Incident radiation varies throughout the year with highest radiation

(24 $MJ/m^2/d$) occurring in the summer months. Mean annual temperature is 24.6^0 C and ranges from 18 to 30^0 C (Appendix A).

Clones of *L. esculenta* K838 x *Leucaena leucocephala* K636 (a sterile triploid, TRIP), and *L. pallida* K804 (PALL) were used. Plants were grown outdoors and maintained as actively growing hedges. Irrigation at the base of the plant was applied to promote rapid growth. One-half meter long cuttings were taken from three to 12 weeks regrowth, cut to 0.25 m length, put on ice, and transported to the Hawaiian Sugar Planters Association (HSPA) laboratory within 1 hour. Cuttings were soaked in tap water for 10 to 15 minutes, stripped of leaves and cut into pieces 2 to 3 cm in length.

Plant material was harvested four times in a nine-month period. The first group (GROUP I) was taken in December, 1992, the second group (GROUP II) in January, 1993, the third group (GROUP III) in June and the fourth group (GROUP IV) in August, 1993 representative of winter and summer seasons in Hawaii.

The gel was 8 % Difco agar with 1/2 MS salts (Murashige and Skoog, 1962). Chemical composition of the gel is listed in Table 9.1. The 50 % reduction in mineral salts was chosen based on preliminary observations at HSPA by Austin and Harris (data not published) that full MS gels promoted an over-abundance of callus tissue in *Leucaena* cuttings.

Table 9.1. Chemical composition of gel† for micropropagation of *Leucaena* spp. explants.

Compound	Quantity
Murashige and Skoog (MS) salt	2.15 g l^{-1}
Sucrose	30 g l^{-1}
Thiamine HCl	0.1 mg l^{-1}
Myoinositol	100 mg l^{-1}
BA (N-6 Benzyladenine)	3.0 mg l^{-1}
NAA (napthaleneacetic acid)	0.05 mg l^{-1}

†gel pH adjusted to 5.8 using either 1N HCl or 1N NaOH.

Variations of the sterilization techniques were derived from earlier experimentation at HSPA by Austin and Harris (data not published) in which varying success rates led to the formulation of these studies.

GROUP I was designed to determine if a drying down period after dipping explants in EtOH facilitated sterilization. Explants were 6 week old TRIP and PALL regrowth. A total of 28 samples (14 in each dry down period) of each genotype were used. All explants were sterilized using a 3 second dip in 90 % EtOH, a 5 minute dip in 20 % v/v Chlorox bleach, 3 rinses in sterile distilled water, a 5 minute dip in 0.1 % mercuric chloride ($HgCl_2$) and 3 rinses in sterile distilled water. Two drying down periods of 5 and 15 minutes after dipping in EtOH were used prior to placing the explants in 1/2 MS gel.

GROUP II was designed to test various deviations on the standard sterilization procedure. Explants were 9 week old TRIP regrowth placed under five separate sterilization procedures. Sterilization techniques A-E are listed in Table 9.2. Procedure A started with had 20 explants, Procedure B had 19 explants, Procedure C had 13 explants, Procedure D had 13 explants, and Procedure E had 20 explants.

GROUP III was designed to examine variations in age of plant regrowth on sterilization success. TRIP explants were selected with regrowth intervals of 3, 6, 9 and 12 weeks.

Table 9.2. Description of GROUP II sterilization procedures.

Procedure	Sterilization Technique†
Procedure A	3 s dip in EtOH, 5 minute dip in 1 % CTAB, rinse twice, 10 minute dip in 5 % chlorox bleach, rinse three times.
Procedure B	3 s dip in EtOH, 5 minute dip in 1 % CTAB, rinse twice, 10 minute dip in 5 % chlorox bleach, rinse three times, 5 minute dip in 0.1 % $HgCl_2$, rinse three times.
Procedure C	3 s dip in EtOH, 5 minute dry down, 5 minute dip in 1 % CTAB, rinse 3 times, 15 minute dip in 3 % chlorox bleach, rinse three times.
Procedure D	3 minute soak in liquid detergent (Joy), 10 minute dry down, 3 s dip in EtOH, 10 minute dip in 10 % chlorox bleach, rinse three times.
Procedure E	3 minute soak in liquid detergent (Joy), 10 minute dry down, 3 s dip in EtOH, 20 minute dip in 20 % chlorox bleach, rinse three times.

†Explants in procedures A-E were rinsed in sterile distilled water.

A total of 15 samples per growth interval were placed into test tubes filled with 1/2 MS gel. The sterilization procedure was a 3 second dip in 90 % EtOH, a 10 minute dip in 20 % v/v Chlorox bleach, 3 rinses in sterile distilled water, a 10 minute dip in 0.1 % mercuric chloride ($HgCl_2$) and 3 rinses in sterile distilled water.

GROUP IV was used to fine tune the success observed from GROUP III sterilization procedures. Twenty-eight explants of seven week regrowth of both TRIP and PALL explants were placed in test tubes containing 1/2 MS gel. The sterilization procedure used was 3 second dip in 90 % EtOH, a 7 minute dip in 20 % v/v Chlorox bleach, 3 rinses in sterile distilled water, a 7 minute dip in 0.1 % mercuric chloride ($HgCl_2$) and 3 rinses in sterile distilled water.

Explants from each GROUP were placed in continuous lighting at 35 μmol m^{-2} s^{-1} with temperature of 28-30 ^{0}C. Explants were checked for cleanliness after 2 and 4 weeks.

Infected explants were examined by Dr. George Wong, (Assoc. Prof., Dept. of Botany, University of Hawaii) for external and internal pathogens.

RESULTS and DISCUSSION

The highest sterile explant percentages came from GROUP III and GROUP IV sterilization modifications ranging from 36 to 80 % success (Table 9.3). These procedures were similar to what Goyal *et al.* (1985) reported for *L. leucocephala* K67

except they sterilized explants from greenhouse grown material. No sterilized explants were observed from procedures reported by Dhawan and Bhojwani (1985).

All explants in GROUP I were either contaminated or died as the result of over-sterilization after 2 weeks (Table 9.3). Both internal and external pathogens were observed on the explants and in the gel. Pathogens identified included *Penicillium* spp., *Colletotrichum* (external), and *Fusarium* spp. (internal).

GROUP II sterilization Procedures A, B, and C yielded no sterile explants after 23 days, while explants in Procedures D and E produced 15 and 35 % clean material, respectively (Table 9.3). Variations on the Dhawan and Bhojwani (1985) procedure were ineffective in sterilizing explants. It is not clear why Procedures D and E of GROUP II had several clean explants. Sterilization techniques were somewhat similar with the exception that CTAB was replaced by liquid Joy or a commercially available dish detergent.

In contrast to GROUP I and II explants, 60 % of all explants in the GROUP III study were healthy after 28 days. Age of plant material did not affect the number of sterile explants obtained (Table 9.3). GROUP IV also produced higher numbers of sterile explants with 36 % of the PALL and 54 % of the TRIP explants clean after 23 days (Table 9.3).

Table 9.3. Number of clean, living *Leucaena* explants† out of total number tested in GROUPS I-IV in Hawaii.

Group #		TRIP	% Clean	PALL	% Clean
I	(5 min dry down)	0/14		0/14	
	(15 min dry down.)	0/14		0/14	
II	**(Proc. A)**	0/20		N/A‡	
	(Proc. B)	0/19			
	(Proc. C)	0/13			
	(Proc. D)	2/13	(15 %)		
	(Proc. E)	7/20	(35 %)		
III	(3 wks regrowth)	7/15	(47 %)	N/A‡	
	(6 wks regrowth)	12/15	(80 %)		
	(9 wks regrowth)	6/15	(40 %)		
	(12 wks regrowth)	11/15	(73 %)		
IV		15/28	(54 %)	10/28	(36 %)

†Explants used were the *L. leucocephala* K636 x *L. esculenta* K838 (Triploid), and *L. pallida* K804.
‡N/A explants from this source were not used in groups II and III.

Both GROUP I and II explants were taken in winter weather which may explain the low sterilization success of these explants, while GROUP III and IV explants were taken in summer. Environment appears to play a critical role for successful sterilization of *Leucaena*. Winter weather at Waimanalo consists of decreased solar radiation, cool temperatures and high rainfall (Appendix A). These conditions slow plant growth (Brewbaker *et al.*, 1972; Evensen, 1985) and are more favorable for pathogens. In contrast, summer has higher incident solar radiation, warmer temperatures, and longer periods without significant rainfall for up to 2 weeks. These conditions promote rapid growth of irrigated *Leucaena*. Summer climates thus decrease the likelihood of internal pathogen infection of the shoot tip since the vascular system has not yet been developed. These conditions also provide vigorous explant material void of any bark or other hardened layers that may trap and protect external pathogens.

CONCLUSION

Sterilization of *Leucaena* explants is possible from plants growing outdoors in Hawaii. Variations of the Goyal *et al.* (1985) technique are best when rapidly growing coppiced material from material taken from the middle third of the cutting is used. In order to increase sterilization success rates, summer-grown barkless explants should be

used. Explants from the tip of the plant are often burned in the sterilizing solution, while explants taken further down have too much woody material and a more developed vascular system to promote high sterilization rates.

CHAPTER 10

VEGETATIVE PROPAGATION OF *LEUCAENA* SPECIES

ABSTRACT

Developing vegetatively propagated *Leucaena* spp. will aid toward developing F_1 hybrid seed production by capitalizing on self-incompatible species within the genus. This research was conducted to clone improved *Leucaena* for use in seed production orchards and biomass production trials. We also wanted to determine if cooler,cloudier climates are more favorable for cloning *Leucaena*, and whether high tannin concentrations were responsible for rooting inhibition. Forty *Leucaena* species and hybrid cuttings 1 m long were taken from coppiced field plants at Waimanalo, Hawaii, four times from Nov. 1993 to April 1994. Explants were cut into 6 to 10 cm long pieces approximately 10 mm in dia. containing one bud and a single clipped leaf, these were then dipped in rooting hormone, and placed into Oasis wedges under shade cloth and intermittent mist. Sixty-six percent (27 out of 40) of the genotypes tested rooted within 4 to 5 weeks. Rooting percentage within genotypes ranged from 7 to 60 %. Rooted explants were repotted and remained in the greenhouse for two weeks after which they were removed and hardened outside for an additional two week period. All cuttings survived and were transplanted between May and June 1994. Mortality after

transplanting was low with only six out of 53 clones (11 %) dying. Clones will be used for seed production orchards in Hawaii.

INTRODUCTION

Leucaena leucocephala is difficult to clone by conventional methods of vegetative propagation (NRC, 1977). Previous attempts at cloning *Leucaena* using the techniques of Hu and Liu (1981), and Bristow (1983) have met with little success in Hawaii. Hu and Liu (1981) report a high level of success, in some cases up to 100 % after placing small 3-leaf *L. leucocephala* cuttings in course sand medium under intermittent mist in Taiwan. Bristow (1983) reported varying levels of success using *L. leucocephala* cuttings. In particular, one experiment used cuttings that had been taken from trees grown in the greenhouse, wilted for 60 minutes, and then placed under polyethylene to maintain high relative humidity and temperature (27 ^{0}C).

Vegetative propagation techniques that are effective in other nitrogen-fixing genera include root propagation of *Robinia pseudoacacia* L. (Bujtas and Papp, 1988), stem cuttings of *Acacia auriculiformis*, and *A. mangium* (Ahmad, 1992; Badji *et al.*, 1991; Hu and Shen, 1986), and sprig cuttings of *Casuarina* spp. (Lundquist and Torrey, 1984). A few legume trees root with ease from large stem cuttings, such as *Gliricidia sepium* (Duguma, 1988) and *Erythrina* spp.

Successful vegetative propagation of *Leucaena* spp. may be highly dependent on variations in temperature and humidity, and affected by the source of plant material utilized. Cool, humid conditions may enhance rooting. Pal (1993) reported improved rooting ability of hardwood cuttings of *L. leucocephala* from March to August in Dehra Dun, India after soaking in 200 mg L^{-1} IBA (Indolebutyric acid) for 24 hours. Hu and Liu (1981) and Bristow (1983) both conducted their experiments in cooler climates and with greenhouse-grown plants.

Improved cloning techniques will allow the development of clonal cross-pollinated F_1 hybrid seed orchards by capitalizing on the self-incompatibility complex found in *L. pallida* lines (Brewbaker, 1982). Clones can also be used to develop homogeneous pure-lines, or to propagate sterile triploids such as *L. esculenta* x *L. leucocephala*. Clones can be transferred to isolated sites and form part of a nucleus breeding strategy (Cotterill *et al.*, 1990). Production of hybrid plants superior to local *L. leucocephala* (common leucocephala) will increase consumer awareness of alternative *Leucaena* spp.

The objective of this experiment were to develop appropriate techniques for clonally propagating leucaena, in contrast to the more laborious and costly method of micropropagating *Leucaena*. The importance of this research lies in the need to produce hybrid progeny from self-

incompatible *L. pallida* lines. Superior trees identified in the field are to be clonally propagated after coppicing and used in seed orchards.

MATERIALS AND METHODS

Forty individual *Leucaena* spp. and hybrids were selected based on superior growth or combining ability for hybrid production (Table 10.1). All parent plants were growing at the Waimanalo Research Station, Oahu, Hawaii and were either managed in forage hedgerows or coppiced prior to taking cuttings. Cuttings approximately 0.5 to 1 m long were taken on 14 November 1993, and 27 January, 7 April and 14 April 1994 and placed in 10 L plastic buckets with water. They were transported several miles to greenhouses at Maunawili, Oahu (elevation 200 m) and cut into 6 to 10 cm pieces approximately 6 to 10 mm in diameter. Individual cuttings had a single clipped leaf and one bud centered in the midsection. Cuttings were dipped into rooting hormone (Hormodin number 3) for hardwood cuttings, fitted into Oasis wedges, and placed under 40 % shade cloth with misting intervals of 4 minutes and a 4 second duration. Rooted cuttings were transferred to 10 cm plastic pots filled with number 4 Sunshine Mix (Fison LtD, Alberta, Canada) and grown for 2 weeks in the greenhouse. Cuttings were placed outdoors for approximately 2 to 3 weeks to harden prior to

Table 10.1. *Leucaena* species and hybrids studied for vegetative propagation and number of plants successfully rooted from cuttings treated 5 to 6 weeks previously with hormodin number 3.

Genotype	Total Rooted†
14 November 1993	
L. esculenta x *L. leucocephala* K838 x K636 F_1	6/10
L. pallida x *L. leucocephala* K748 x K636 F_1	1/10
L. pallida x *L. leucocephala* K806 x K636 F_1	4/10
L. diversifolia x *L. leucocephala* K156 x K636 F_2	3/10
L. pallida x *L. leucocephala* K804 x K636 F_1	0/10
L. pallida x *L. pallida* K748 x K806 F_1	0/10
L. pallida K376	0/22
L. pallida K804	0/10
L. diversifolia K749 (2x)	0/10
AVERAGE	14/102
27 January 1994	
L. pallida x *L. leucocephala* (K376 x K8) F_4	5/12
L. pallida K748 OP	2/12
L. diversifolia K784 (4x)	4/10
L. diversifolia K785 (4x)	1/7
L. pallida x *L. leucocephala* K748 x K584 F_1	0/10
L. pallida x *L. leucocephala* K804 x K636 F_1	0/11
L. pallida x *L. pallida* K748 x K806 F_1	0/10
L. pallida K376	0/12
L. pallida K806 OP	0/12
L. diversifolia x *L. leucocephala* K156 x K636 F_3	0/11
AVERAGE	12/107

Table 10.1, Continued

7 April 1994

L. pallida x *L. leucocephala* K748 x K636 F_1	3/14
L. pallida x *L. leucocephala* K748 x K584 F_1	1/28
L. pallida x *L. leucocephala* K748 x K481 F_1	4/14
(*L. leucocephala* x *L. diversifolia*) x *L. pallida* (K636 x K156) x K806	1/14
(*L. leucocephala* x *L. diversifolia*) x *L. pallida* (K636 x K156) x K748	2/14
L. pallida x *L. pallida* K806 x K748 F_1	2/14
L. diversifolia x *L. leucocephala* 91-12 F_3	2/14
L. diversifolia x *L. leucocephala* 91-13 F_3	6/14
L. pallida x *L. leucocephala* K376 x K8 F_4 3-32 OP	7/14
L. pallida K748 sib	1/12
L. leucocephala K420	1/14
L. pallida K953	0/14
AVERAGE	36/180

14 April 1994

L. pallida x *L. leucocephala* K376 x K8 F_3 3-30	6/12
L. pallida x *L. leucocephala* K376 x K8 F_3 5-17	4/11
L. pallida x *L. leucocephala* K376 x K8 F_3 6-16	3/10
L. pallida x *L. leucocephala* K376 x K8 F_4 3-32 OP	3/13
L. pallida x *L. leucocephala* K376 x K8 F_4 5-36 OP	2/11
L. pallida x *L. leucocephala* K376 x K8 F_4 7-13 OP	3/12
L. pallida x *L. leucocephala* K376 x K8 F_4 6-9 SELF	1/10
L. pallida x *L. leucocephala* K376 x K8 F_4 6-34 SELF	2/10
L. pallida x *L. leucocephala* K376 x K8 F_4 6-15 OP	0/10
L. pallida x *L. leucocephala* K376 x K8 F_4 7-5 SELF	0/10
AVERAGE	24/109

†Total number of explants that rooted after 4 to 5 weeks over the total number of explants tested.

transplanting to the field. Cuttings were transplanted on 4 May and 14 June 1994 and tended as nurse plants for future clonal propagation.

RESULTS

Twenty-seven of the forty lines (66 %) developed roots within 5 to 6 weeks (Table 10.1). Rooting percentage within successfully rooted lines ranged from 7 to 60 %. In all, a total of 25 *Leucaena* species and hybrid clones from this study were developed and are growing at Waimanalo.

Although not tested, it appeared that cuttings taken from the second node and third nodes from the top were best for developing roots. Explants from the top of the cutting usually wilted and died before setting roots.

The most difficult plants to propagate were *L. pallida* genotypes. *Leucaena pallida* K376, K953, K806 and K804 repeatedly failed to develop roots, while only one cutting each of the K748 sib and K748 OP rooted. Interestingly, the F_1 hybrids of K376 x K8, K748 x K636 and K806 x K636 and three-way hybrids (K156 x K636) x K748 and (K156 x K636) x K806 developed roots. The highest rooting percentage belonged to *L. esculenta* x *L. leucocephala* K838 x K636 F_1 clones developed prior to this study via an alternative propagation technique. *Leucaena pallida* K804 clones tested at the same time as the triploid clone did not root in this study. Ten out of 12 advanced progeny KX2 hybrid genotypes

based on the parentage *L. pallida* K376 x *L. leucocephala* K8 rooted, while all three advanced KX3 progeny *L. diversifolia* K156 x *L. leucocephala* K636 genotypes rooted. Establishment success after transplanting to the field was high with only 6 individual clones out of 53 (11%) dying.

Clones K806 x K636 F_1, K748 x K636 F_1, KX2 sc and K784 transplanted in May 1994 ranged from 1 to 2 m in height after 3 months.

DISCUSSION

The wide range of *Leucaena* species and hybrid rooting percentage in this study reflects the diversity of the material tested, and is within published accounts from both Bristow (1983) and Hu and Liu (1981). Lower intrinsic rooting was associated with *L. pallida* genotypes, while the higher rooting percentages were represented by F_1 hybrids between *L. pallida* and *L. leucocephala*.

Root initiation may be inhibited by phenolic compounds (Hartmann *et al.*, 1990, p. 209). Soluble polyphenol concentrations (tannins) were examined in many of these same *Leucaena* lines in a separate experiment (Austin, Chapter 3) which suggests that tannins were not responsible for affecting root strike. However, this particular assay is a general test for phenolics. Individual phenolic compounds were not elucidated, and it is possible that one of these are responsible for root inhibition.

While average *L. leucocephala* tannin concentrations were the lowest among 11 genotypes tested with 96 g kg^{-1}, (Table 10.2) they were not significantly different from K748 OP, K806 OP, or K748 x K806 averaging 108 mg g^{-1} (Table 10.2). Tannin concentrations also do not explain why *L. diversifolia* K749 with an average tannin concentration of 100 mg g^{-1}, failed to root, while KX2 (K376 x K8) with a tannin concentration of 112 mg g^{-1} rooted profusely.

The higher rooting percentage of the *L. pallida* x *L. leucocephala* hybrids suggests that *L. leucocephala* promotes rooting. While the only *L. leucocephala* genotype (K420) examined in this study had very low rooting percentage, the study by Hu and Liu (1981) and Bristow (1983) suggests that *L. leucocephala* roots at high levels. Better rooting of the F_1 may enable breeders with hard to root *Leucaena* genotypes to first hybridize these lines with *L. leucocephala*, clone the F_1 hybrid, and then use a series of backcrosses to return to the desired parent while maintaining the ability to root. A specific drawback to this approach is that it may affect the self-incompatibility complex associated with *L. pallida* through competition interaction (Brewbaker, 1982) and negate the advantage that self-incompatibility has to offer in developing cross-pollinated progeny.

The high rooting percentage of the clone *L. esculenta* x *L. leucocephala* F_1 suggests that large quantities of ramets can be made available from several cloned ortets.

Table 10.2. Average soluble tannin concentrations mg g^{-1} of some *Leucaena* genotypes at Waimanalo, Hawaii.

Genotype†	Tannin Conc. mg g^{-1}
L. pallida x *L. leucocephala* K748 x K636 F_1	103
L. pallida x *L. leucocephala* K748 x K584 F_1	112
L. pallida x *L. pallida* K748 x K806 F_1	112
L. pallida x *L. leucocephala* K806 x K636 F_1	104
L. pallida x *L. leucocephala* K376 x K8 F_4	112
L. pallida K748 sib	101
(*L. leucocephala* x *L. diversifolia*) x *L. pallida* (K636 x K156) x K806	115
L. diversifolia K784 (4x)	113
L. pallida K806 OP	110
L. diversifolia x *L. leucocephala* K156 x K636 F_3	110
L. leucocephala K636	96
L. diversifolia K785 (4x)	111
L. diversifolia (2x)	100
L. pallida K376	99
L. pallida x *L. leucocephala* K804 x K636 F_1	101

†Genotypes from this study and the forage production study are from the same field SET 93-3 at Waimanalo, Hawaii.

However, the poor rooting performance of ramets from the *L. pallida* K804 clone casts doubt on the practice of producing clones from cloned mother plants. The lack of rooting of K804 may be due to seasonal variation or because a sample size was too small. The high percentage of establishment of transplanted clones in the field is reassuring for developing seed orchards, clonal biomass production schemes and for developing classical genotype by environment interactions.

CONCLUSIONS

The success of *Leucaena* vegetative propagation represents a breakthrough for improving various select genotypes through hybridization or species level improvement schemes. The most difficult genotype to propagate was *L. pallida*, yet it is this species that forms the backbone of future hybridization schemes. Hybridization of *L. pallida* with *L. leucocephala* facilitates rooting and can be used to develop F_1 hybrid, three way crosses with either diploid or tetraploid species (e.g. *L. leucocephala* x *L. pallida*) x *L. diversifolia* (2n or 4n) or backcross lines as long as self-incompatibility is retained. Tannins do not appear to hinder rooting of *Leucaena*.

CHAPTER 11

SUMMARY

To summarize 10 separate chapters is difficult, yet necessary in order to weave a thread through all the data and produce recommendations to guide future research.

The high yields, good psyllid resistance and enhanced adaptability of the F_1 hybrids in this study is probably the result of their amphiploid makeup. In the case of the F_1 hybrids between *L. pallida* by *L. leucocephala* their chromosomal makeup is thought to be from 4 separate genomes (Pan, 1985; Pan and Brewbaker, 1988). *Leucaena pallida* is thought to be a naturally occurring hybrid between *L esculenta* and *L. diversifolia* ssp. *trichandra*, while *L. leucocephala* is presumed to have developed from *L. shannonii* and *L. diversifolia* ssp. *trichandra* (Pan and Brewbaker, 1988). In any case, improved environmental adaptability would be expected from organisms derived from 4 separate genomes, and gives credence to the overall higher productivity across environments of the F_1 hybrids. Although the extreme yield variation of the F_1 hybrid K806xK636 refutes this observation.

Heritability of psyllid resistance in advanced generations has been shown and the fact that psyllid resistance and vigor were moderately yet signifiacnatly

correlated allows the breeder to concentrate on selecting the best forage varieties based on psyllid resistance alone.

Forage digestibility was lower at Mealani than at Waimanalo which is in contrast to reports that digestibility of grass forages increases in cooler climates (Akin, 1989; Minson and Wilson, 1980). It is not clearly understood why this is except that it is logical to assume that the undigestible fractions of either cellulose or lignin were increased as a result of stress. The positive correlation between IVDMD and tannin concentration at Mealani rules out the possibility that tannins were responsible for decreased digestibility. Another point to consider is why IVDMD assays at Hawaii resulted in higher digestibility (approx. 20 %) than IVOMD assays from Florida on the same selections. In-vitro organic matter digestibility (IVOMD) was tested on K636, KX2 (K376xK636), K376, KX3 (K156xK636) and K948 at Brooksville, FL (Austin *et al.* 1995) using the Tilley and Terry method (1963) while the IVDMD procedure (Goering and Van Soest, 1970) was used at the University of Hawaii. It may be as simple as the differences in the rumen fluid collected from the fistulated steers that caused the differences. Psyllid damage and IVDMD were not correlated at Waimanalo in harvest 1 because psyllid pressure was not sufficient to cause severe damage. However, if IVDMD from later harvests is to be analyzed it is suggested that these parameters would indeed be correlated as they were in

Florida (Austin *et al.* 1995). The next logical step therefore is to develop forage trials that interface with animal preference and liveweight gain studies while scoring for psyllid dmage.

The lack of a negative correlation between tannin concentration and psyllid resistance has confirmed Castillo's (1994) earlier findings. In fact the positive correlation that was observed at harvest 2 at Waimanalo helps rule out tannins as the agent that causes psyllid resistance.

The large variation in *L. leucocephala* yields and psyllid resistance in both SET 83-5 and SET 91-3 confirms the theory that variation exists within the species *L. leucocephala*. This finding is backed by isozyme analyses conducted by Sun (1992) who determined that 75 % of the *L. leucocephala* giants types showed polymorphisms at 4 of the 6 isozyme tested. These observations represent a breeding strategy that could eliminate inbreeding depression by hybridizing the top *L. leucocephala* species observed in these and other trials. Another interesting observation to emerge from this study is that early height measurements may not be a reliable indicator for final tree height. As presented in Chapter 8, K636 started off slowly being outgrown by *L. macrophylla* (K902), *L. lanceolata* (K952), *L. shannonii* (K925), and *L. diversifolia* (K784) in the first six months of height measurements.

The development of vegetative propagation of *Leucaena* is an important finding derived from this study. Clonal propagation will enable breeding programs to develop seed production orchards based on superior cloned material. F_1 hybridization of superior KX2 lines is within reach since *L. pallida* is self-sterile and requires cross-pollination. At the present time, *L. pallida* selections are still difficult to root but the hybrid *L. pallida* by *L. leucocephala* has been successfully rooted. This suggests that *L. leucocephala* promotes rooting and will enable breeders to capture psyllid resistance and seedling vigor from *L. pallida* while developing lines that are morphologically similar to *L. leucocephala*. Success of rooting appears to be due to cooler temperatures and cloudy conditions. Rooting efficiency may be further enhanced by providing heat to the rooting zone. The success of the micropropagation sterilization study indicates that hard to root *Leucaena* species may be rooted using micropropagation. The real importance of this finding is that hard to root, superior field-grown material may be cloned thus capturing select genotypes for further breeding. Sterilization of *Leucaena* micropropagules is improved when explants are taken from rapidly growing, barkless material grown under the high light and low rainfall conditions of summer indicating that internal pathogens are partially responsible for sterilization failure.

Practical applications from this study include several recommendations. In terms of forage the strategies must now include livestock measurements such as preference, liveweight gains, and stocking rates. Forage quality, quantity and psyllid resistance are have shown to be adequate for ruminant production standards. Also the new F_1 hybrids like K748xK636 and K748xK584 have proven their ability to outperform the standard *L. leucocephala* checks across diverse environments. The best way to approach this is to provide F_1 hybrids, parents and the first and second generation backcrosses to feeding trials. Results from this study will assist the overall seed production strategy currently being pursued by the Hawaiian Sugar Planters Association and the University of Hawaii by keying on the best varieties for animal production.

In terms of developing hybrids it is suggested that KX2 be developed from *L. pallida* K748 and *L. leucocephala* K636 or K584. Also the advanced progeny KX2 and KX3 hybrids are important since they are proven seed resources based on superior plant material. Hybrid KX3 should be developed from another *L. diversifolia* parent. Results show that K784 has better psyllid resistance and wood yields than K156. Finally, triploid hybrids should also be given fair consideration since they can now be vegetatively propagated, and seed sterile.

APPENDIX A

Weather data for rainfall, temperature and solar radiation at Waimanalo Research Station, and temperature data from the Mealani Research Station.

Table A.1. Rainfall (mm) at Waimanalo, Hawaii from 1989 to 1994.

Month	1989	1990	1991	1992	1993	1994
January	89	242	108	40	39	77
February	221	160	92	60	32	33
March	135	141	M†	49	2	M
April	315	8	65	40	21	23
May	24	37	54	148	23	23
June	47	17	23	21	14	22
July	89	41	9	74	54	
August	48	17	41	37	33	
September	26	32	100	83	15	
October	280	33	240	223	68	
November	83	192	107	275	22	
December	123	115	107	263	21	
Total	1481	1035	946	1313	344	

†M is missing data.

Table A.2. Mean temperature (^{0}C) at Waimanalo, Hawaii from 1989 to 1994.

Month	1989	1990	1991	1992	1993	1994
January			22.2	22.1	22.5	22.9
February			22.4	22.6	21.0	21.0
March			21.9	M†	24.5	M
April			23.6	23.3	25.5	27.0
May			24.4	24.8	25.8	25.8
June			25.3	26.4	27.7	25.2
July			26.0	27.0	27.4	
August			26.4	M	27.5	
September			25.7	26.7	27.6	
October			25.6	26.2	25.7	
November			25.9	25.1	24.8	
December			24.8	24.9	23.8	

†M is missing data.

Table A.3. Solar Radiation (MJ m^{-2} day^{-1}) at Waimanalo, Hawaii from 1989 to 1994.

Month	1989	1990	1991	1992	1993	1994
January			10.5	11.3	12.8	10.2
February			13.1	12.9	16.4	16.4
March			12.4	M†	19.0	M
April			19.9	18.2	20.5	23.4
May			23.1	20.3	22.0	22.0
June			23.7	25.3	24.6	21.5
July			21.9	23.2	24.7	
August			19.1	M	21.4	
September			19.1	20.8	21.5	
October			14.5	15.0	14.3	
November			11.8	11.4	11.9	
December			9.3	9.9	9.8	

†M is missing data.

Table A.4. Average maximum and minimum temperature for the Mealani Research Station† from 1966-1990.

	------- °C -------	
Month	Maximum	Minimum
January	20.0	10.5
February	20.1	10.5
March	19.0	11.0
April	18.0	12.0
May	20.0	12.5
June	20.5	13.5
July	21.0	15.0
August	22.0	15.0
September	22.5	14.0
October	22.0	13.5
November	21.0	12.5
December	20.0	11.0

†The Mealani Research Station is situated at 850 m.a.s.l. on the island of Hawaii, the mean annual rainfall is 1480 mm averaged over 25 years.

APPENDIX B

Average forage biomass production, days to harvest, total growing degree days (GDD), and forage produced per GDD at Waimanalo, Hawaii for GROUP 1 and 2 selections in SET 91-2.

Table B.1. Average forage DM yield, days to harvest, total growing degree days and average daily forage biomass (kg ha^{-1}) production for 5 harvests of Group 1 *Leucaena* varieties.

Parameter	1	2	3	4	5
Avg. (kg ha^{-1}) †	3600	4700	3900	4100	6000
Days to Harv.	104	68	85	73	66
Total GDD‡	1600	1078	1258	921	925
Forage kg ha^{-1} GDD^{-1}§	2.25	4.35	3.10	4.45	6.48

†Based on average forage production of 10 selections.
‡Total Growing degree days in ^0C for the time period between harvests. Based on (Max+Min/2)-10^0.
§Average kg of dry weight per growing degree day based on kg ha^{-1} GDD^{-1}.

Table B.2. Average forage DM yield, days to harvest, total growing degree days and average daily forage biomass production (kg ha^{-1}) for 5 harvests of Group 2 *Leucaena* varieties.

Parameter	1	2	3	4	5
Avg. (kg ha^{-1})†	1300	1500	1700	1500	3300
Days to Harv.	104	68	85	73	66
Total GDD‡	1600	1078	1258	921	925
Forage kg ha^{-1} GDD^{-1}§	0.81	1.39	1.35	1.62	3.56

†Based on average forage production of 21 selections.
‡Total Growing degree days in C^0 for the time period between harvests. Based on (Max+Min/2)-10^0.
§Average kg of dry weight per growing degree day based on kg ha^{-1} GDD^{-1}.

APPENDIX C

List of *Leucaena* selections supplied to the various research stations involved in the Africa Leucaena Psyllid Trial (ALPT).

Table C.1. The Machakos Research Station, Kenya, Africa.

Replicated Entries:

KX1 (Pallida x Diversifolia) K376 x K156 (F_3)
KX2 (Pallida x Leucocephala) K376 x K8 (F_3)
KX3 (Leucocephala x Diversifolia) K636 x K156 (F_3)
K156 Diversifolia (4n)
K784 Diversifolia (4n)
K636 Leucocephala
K584 Leucocephala
K565 Leucocephala
K584 x K636 Leucocephala (F_2)
K376 Pallida
K824 Pallida
K953 Pallida
K748 Pallida
K948 Esculenta (psyllid resistant control)
K997 Leucocephala (psyllid susceptible control)

Unreplicated or Augmented Entries:

KX2 Tree 6-15
KX2 Tree 6-9
KX2 Tree 5-36
KX2 Tree 3-5
KX2 Tree 3-26
KX3 Tree 91-13
K989 Diversifolia 2n
K907 Diversifolia 2n
K806 Pallida
K75 Pulverulenta

Note: 3 month old seedlings transplanted on 5 November 1993.

Table C.2. The Maseno Research Station, Kenya, Africa.

Replicated Entries:

KX1 (Pallida x Diversifolia) K376 x K156 (F_3)
KX2 (Pallida x Leucocephala) K376 x K8 (F_3)
KX3 (Leucocephala x Diversifolia) K636 x K156 (F_3)
K156 Diversifolia (4n)
K784 Diversifolia (4n)
K636 Leucocephala
K584 Leucocephala
K565 Leucocephala
K584 x K636 Leucocephala (F_2)
K376 Pallida
K824 Pallida
K953 Pallida
K748 Pallida
K948 Esculenta (psyllid resistant control)
K997 Leucocephala (psyllid susceptible control)

Unreplicated or Augmented Entries:

KX2 Tree 6-15
KX2 Tree 6-9
KX2 Tree 5-36
KX2 Tree 3-26
KX2 Tree 5-20
KX3 Tree 91-13
K989 Diversifolia 2n
K806 Pallida
K820 Pallida
K75 Pulverulenta

Table C.3. The Makoko Research Station, Malawi, Africa.

Replicated entries:

KX1 (Pallida x Diversifolia) K376 x K156 (F_3)
KX2 (Pallida x Leucocephala) K376 x K8 (F_3)
KX3 (Leucocephala x Diversifolia) K636 x K156 (F_3)
K156 Diversifolia (4n)
K784 Diversifolia (4n)
K636 Leucocephala
K584 Leucocephala
K565 Leucocephala
K584 x K636 Leucocephala (F_2)
K376 Pallida
K824 Pallida
K953 Pallida
K748 Pallida
K948 Esculenta (psyllid resistant control)
K997 Leucocephala (psyllid susceptible control)

Unreplicated or Augmented Entries:

KX2 Tree 6-15
KX2 Tree 6-9
KX2 Tree 5-36
KX2 Tree 3-5
KX2 Tree 3-26
KX3 Tree 91-13
K989 Diversifolia 2n
K907 Diversifolia 2n
K806 Pallida
K820 Pallida

Table C.4. The Arusha Research Station, Arusha, Tanzania.

Replicated Entries:

KX1 (Pallida x Diversifolia) K376 x K156 (F_3)
KX2 (Pallida x Leucocephala) K376 x K8 (F_3)
KX3 (Leucocephala x Diversifolia) K636 x K156 (F_3)
K156 Diversifolia (4n)
K784 Diversifolia (4n)
K636 Leucocephala
K584 Leucocephala
K565 Leucocephala
K584 x K636 Leucocephala (F_2)
K376 Pallida
K824 Pallida
K953 Pallida
K748 Pallida
K948 Esculenta (psyllid resistant control)
K997 Leucocephala (psyllid susceptible control)

Unreplicated or Augmented Entries:

KX2 Tree 6-15
KX2 Tree 6-9
KX2 Tree 5-36
KX2 Tree 3-5
KX2 Tree 3-26
KX2 Tree 5-20
KX3 Tree 91-13
K806 Pallida
K820 Pallida
K450 Collinsii

Note: This trial was directly sown in 1993 with consequent poor establishment. It has been written off as lost by both ICRAF and ILCA.

APPENDIX D

Damage and vigor ratings for 6 observational periods from July 1992 to May 1994 in SET 92-3 and SET 92-4 at Waimanalo, Hawaii.

Table D.1. Damage and vigor ratings + standard errors and rank of open pollinated OP KX2 families on 16 July 1992.

Family	Damage	Rank	Vigor	Rank	
3-3	3.1±0.13	17	2.3±0.09	19	
3-6	3.2±0.14	19	2.8±0.08	3	
3-9	3.0±0.15	16	2.6±0.08	6	
5-3	2.3±0.17	11	2.4±0.10	13	
5-20	2.5±0.11	12	2.9±0.08	1	*
5-36	2.3±0.21	8	2.7±0.09	4	***
6-20	3.6±0.15	22	2.4±0.08	14	
6-34	3.4±0.12	20	2.3±0.07	20	
6-15	1.9±0.11	4	2.2±0.08	21	
4-7	2.3±0.16	9	2.3±0.09	18	
1-3	3.1±0.19	18	2.1±0.08	26	
3-32	2.7±0.19	13	2.8±0.16	2	
3-5	1.9±0.25	5	2.5±0.20	8	***
3-26	3.6±0.14	23	2.6±0.08	5	
5-33	4.0±0.22	26	2.2±0.17	22	
7-8	2.9±0.30	14	2.6±0.14	7	
5-9	4.1±0.16	27	2.1±0.10	24	
2-4	2.9±0.42	15	2.4±0.13	15	
4-13	3.9±0.43	25	2.1±0.13	23	
6-2	3.5±0.46	21	2.4±0.13	16	
6-9	3.6±0.47	24	2.5±0.20	11	
6-28	2.1±0.66	7	1.6±0.24	27	
7-13	2.1±0.29	6	2.5±0.23	9	***
2-9	1.4±0.27	1	2.4±0.15	12	*
3-36	1.5±0.20	2	2.5±0.20	10	***
6-38	2.3±0.25	10	2.1±0.18	25	
1-5	1.8±0.22	3	2.3±0.09	17	
Mean	2.8±0.05		2.5±0.02		
CV	33		21		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top ten finish in both categories of psyllid damage and vigor.

Table D.2. Damage and vigor ratings + standard error and rank of open pollinated OP KX2 families on 20 August 1992.

Family	Damage	Rank	Vigor	Rank	
3-3	2.9±0.18	20	2.3±0.09	24	
3-6	2.8±0.20	19	2.8±0.10	4	
3-9	2.8±0.15	18	2.5±0.09	19	
5-3	1.4±0.11	5	2.3±0.07	23	
5-20	1.3±0.14	4	2.8±0.07	6	***
5-36	1.5±0.15	7	2.8±0.09	5	***
6-20	3.1±0.17	23	2.6±0.07	11	
6-34	2.5±0.15	15	2.4±0.07	21	
6-15	1.3±0.10	3	2.5±0.08	18	
4-7	1.8±0.14	11	2.6±0.08	12	
1-3	2.7±0.17	17	2.5±0.08	20	
3-32	1.8±0.17	10	3.1±0.11	1	*,***
3-5	1.2±0.14	2	2.6±0.20	10	***
3-26	3.3±0.20	26	2.7±0.05	8	
5-33	3.2±0.36	24	2.4±0.15	22	
7-8	2.3±0.30	13	2.9±0.16	3	
5-9	3.0±0.28	21	2.2±0.13	27	
2-4	2.4±0.24	14	2.5±0.00	17	
4-13	4.0±0.41	27	2.5±0.25	15	
6-2	2.5±0.61	16	2.5±0.00	16	
6-9	3.0±0.00	22	2.6±0.13	9	
6-28	2.0±0.71	12	2.3±0.14	25	
7-13	1.8±0.35	9	3.1±0.30	2	***
2-9	1.8±0.48	8	2.8±0.17	7	***
3-36	3.3±0.75	25	2.5±0.00	14	
6-38	1.5±0.26	6	2.2±0.26	26	
1-5	1.7±0.16	1	2.5±0.17	13	*
Mean	2.2±0.05		2.6±0.02		
CV	43		21		

*Denotes the best ranking for either psyllid damage or vigor
***Denotes a top ten finish in both categories of psyllid damage and vigor.

Table D.3. Damage and vigor ratings + standard errors and rank of open pollinated OP KX2 families on 18 October 1992.

Family	Damage	Rank	Vigor	Rank	
3-3	2.1±0.17	16	3.1±0.17	24	
3-6	1.9±0.18	10	4.6±0.17	3	***
3-9	2.4±0.17	18	3.9±0.18	12	
5-3	1.3±0.08	2	3.5±0.14	18	
5-20	1.5±0.13	4	4.3±0.13	5	***
5-36	1.5±0.16	5	4.2±0.15	6	***
6-20	2.1±0.16	12	3.9±0.15	11	
6-34	2.1±0.14	14	3.7±0.15	17	
6-15	1.3±0.13	3	4.0±0.16	10	
4-7	2.1±0.19	13	4.0±0.21	8	
1-3	2.8±0.20	21	3.4±0.13	20	
3-32	1.6±0.20	7	4.7±0.11	2	***
3-5	1.2±0.11	1	3.8±0.31	16	*
3-26	3.5±0.22	26	4.0±0.17	9	
5-33	3.6±0.46	27	3.4±0.34	22	
7-8	1.8±0.27	9	4.6±0.22	4	***
5-9	2.9±0.26	22	3.2±0.32	23	
2-4	3.4±0.24	24	3.1±0.13	25	
4-13	3.4±0.63	25	3.0±0.46	27	
6-2	2.4±0.38	20	3.4±0.24	21	
6-9	2.4±0.55	19	3.9±0.55	14	
6-28	2.3±0.48	17	3.0±0.35	26	
7-13	2.0±0.50	11	4.9±0.48	1	*
2-9	1.7±0.49	8	3.9±0.33	13	
3-36	3.0±2.00	23	3.5±0.50	19	
6-38	1.6±0.26	6	3.9±0.43	15	
1-5	2.1±0.36	15	4.1±0.25	7	
Mean	2.0±0.05		3.9±0.04		
CV	50		25		

*Denotes the best ranking for either psyllid damage or vigor
***Denotes a top ten finish in both categories of psyllid damage and vigor.

Table D.4. Damage and vigor ratings + standard errors and rank of open pollinated OP KX2 families on 24 August 1993. After the first coppice of 27 July 1993 and before rogue number 3.

Family	Damage	Rank	Vigor	Rank	
3-3	2.8±0.23	25	2.2±0.29	25	
3-6	2.4±0.18	17	3.7±0.21	11	
3-9	2.7±0.21	22	3.8±0.24	9	
5-3	2.3±0.39	15	3.4±0.27	20	
5-20	2.3±0.23	14	2.7±0.23	12	
5-36	1.7±0.19	6	3.8±0.17	8	***
6-20	2.5±0.23	19	3.4±0.19	19	
6-34	2.1±0.18	13	3.9±0.18	5	
6-15	1.7±0.18	5	3.9±0.19	6	***
4-7	2.1±0.28	12	3.6±0.34	16	
1-3	3.9±0.28	27	2.7±0.33	22	
3-32	1.4±0.16	3	4.9±0.20	1	*,***
3-5	1.9±0.36	9	3.6±0.35	14	
3-26	2.7±0.24	20	3.8±0.26	7	
5-33	2.7±0.49	21	1.8±0.49	27	
7-8	2.0±0.22	11	4.1±0.35	3	
5-9	2.8±0.24	24	3.5±0.50	18	
2-4	2.4±0.47	16	2.6±0.63	24	
4-13	3.0±0.58	25	2.2±0.93	26	
6-2	2.8±0.25	23	3.5±0.29	17	
6-9	1.8±0.75	7	3.8±0.75	10	***
6-28	2.5±0.29	18	2.6±0.52	23	
7-13	1.5±0.50	4	3.9±0.43	4	***
2-9	1.3±0.33	2	3.7±0.33	13	
3-36	1.0±0.00	1	4.5±0.00	2	
6-38	1.8±0.26	8	3.6±0.49	15	
1-5	1.9±0.35	10	3.4±0.46	21	
Mean	2.3±0.06		3.6±0.04		
CV	41		29		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top ten finish in both categories of psyllid damage and vigor.

Table D.5. Damage and vigor ratings + standard errors and rank of open pollinated OP KX2 families on 28 March 1994. After the second coppice of the trial and before rogue number 4.

Selection	Damage	Rank	Vigor	Rank	
3-3	2.2±0.21	19	2.6±0.64	25	
3-6	1.6±0.20	12	3.7±0.37	15	
3-9	1.7±0.14	13	3.6±0.32	17	
5-3	1.2±0.13	6	3.1±0.38	21	
5-20	1.7±0.12	14	4.0±0.29	13	
5-36	1.1±0.10	3	5.0±0.30	6	***
6-20	1.3±0.17	9	3.7±0.47	16	
6-34	1.2±0.11	7	3.5±0.27	18	
6-15	1.2±0.13	5	4.7±0.30	8	***
4-7	1.8±0.20	16	4.0±0.37	13	
1-3	2.0±0.26	17	2.9±0.66	22	
3-32	1.1±0.07	2	5.3±0.27	3	***
3-5	1.2±0.17	4	4.5±0.32	10	***
3-26	1.4±0.18	10	4.9±0.27	7	***
5-33	1.0±0.00	1	2.5±0.50	24	
7-8	1.1±0.14	3	5.1±0.28	4	***
5-9	1.3±0.16	8	4.6±0.69	9	***
2-4	1.5±0.50	11	3.5±0.50	18	
4-13	1.0± .	1	5.5± .	2	
6-2	1.0±0.00	1	3.3±0.33	19	
6-9	1.8±0.75	15	4.3±0.75	11	
6-28	2.0±0.00	18	3.3 0.25	20	
7-13	1.0±0.00	1	5.5±0.32	2	***
2-9	1.3±0.33	9	5.7±0.33	1	***
3-36	1.0± .	1	5.0± .	5	
6-38	1.1±0.14	3	3.9±0.40	14	
1-5	1.5±0.29	11	4.1±0.83	12	
Mean	1.4±0.04		4.2±0.10		
CV	35		28		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top ten finish in both categories of psyllid damage and vigor.

Table D.6. Damage and vigor ratings + standard errors and rank of open pollinated OP KX2 families on 1 May 1994. After the second coppice of the trial and after rogue number 4.

Family	Damage	Rank	Vigor	Rank	
3-3	1.5±0.29	9	3.6±0.75	22	
3-6	1.8±0.23	14	4.2±0.42	17	
3-9	1.8±0.14	15	3.9±0.40	20	
5-3	1.1±0.07	2	3.7±0.37	23	
5-20	1.7±0.18	12	4.8±0.26	12	
5-36	1.1±0.11	4	5.1±0.28	8	***
6-20	1.1±0.10	3	4.6±0.43	13	
6-34	2.1±0.27	17	3.9±0.27	19	
6-15	1.2±0.09	6	4.8±0.29	11	
4-7	1.1±0.14	5	4.5±0.38	14	
1-3	2.8±0.16	19	4.2±0.73	16	
3-32	1.3±0.11	8	5.7±0.14	1	***
3-5	1.3±0.25	7	4.9±0.31	10	***
3-26	2.4±0.39	18	5.2±0.26	7	
5-33	rogued out of trial				
7-8	1.6±0.33	11	5.2±0.33	6	
5-9	1.7±0.31	13	5.5±0.20	3	
2-4	1.0± .	1	3.5± .	23	
4-13	2.0± .	16	5.5± .	4	
6-2	1.0±0.00	1	3.5±0.00	25	
6-9	2.0±0.00	16	4.3±0.75	15	
6-28	1.0± .	1	3.5± .	24	
7-13	1.0±0.00	1	5.5±0.32	4	***
2-9	1.0±0.00	1	5.5±0.33	2	***
3-36	1.0± .	1	5.0± .	9	
6-38	1.6±0.28	10	3.9±0.4	21	
1-5	1.8±0.75	14	4.1±0.75	5	
Mean	1.49±0.05		4.54±0.09		
CV	38		19		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top ten finish in both categories of psyllid damage and vigor.

Table D.7. Damage and vigor ratings + standard error and rank of self pollinated SELF KX2 families on 16 July 1992.

Family	Damage	Rank	Vigor	Rank	
1-3	2.7±0.14	6	2.1±0.06	10	
6-9	1.1±0.18	1	2.9±0.14	2	***
6-15	1.8±0.11	3	2.6±0.09	6	
7-5	1.5±0.12	2	3.0±0.08	1	***
5-3	2.0±0.15	4	2.5±0.09	7	
6-2	3.2±0.13	11	1.9±0.16	12	
2-4	3.1±0.14	10	2.4±0.08	9	
3-26	3.0±0.17	8	2.5±0.12	8	
3-6	4.0±0.35	12	2.0±0.12	11	
5-36	3.1±0.13	9	2.6±0.13	5	
5-20	2.3±0.32	5	2.6±0.13	4	
6-34	2.9±0.43	7	2.8±0.14	3	
Mean	2.1±0.07		2.5±0.04		
CV	33		21		

***Denotes a top three finish in both categories of psyllid damage and vigor.

Table D.8. Damage and vigor ratings + standard error and rank of self pollinated SELF KX2 families on 20 August 1992.

Family	Damage	Rank	Vigor	Rank	
1-3	2.5±0.19	9	2.4±0.06	11	
6-9	1.0±0.00	1	3.2±0.10	3	***
6-15	1.3±0.12	3	3.0±0.06	4	
7-5	1.1±0.06	2	3.4±0.07	1	***
5-3	1.5±0.15	4	2.9±0.07	5	
6-2	2.8±0.38	12	2.4±0.07	10	
2-4	2.6±0.25	10	2.7±0.08	8	
3-26	2.7±0.21	11	2.8±0.13	7	
3-6	2.5±0.64	8	2.3±0.14	12	
5-36	2.3±0.14	5	2.8±0.14	6	
5-20	2.3±0.48	6	2.6±0.13	9	
6-34	2.5±0.28	7	3.3±0.14	2	
Mean	1.7±0.07		2.9±0.04		
CV	25		15		

***Denotes a top three finish in both categories of psyllid damage and vigor.

Table D.9. Damage and vigor ratings + standard error and rank of self pollinated SELF KX2 families on 18 October 1992.

Selection	Damage	Rank	Vigor	Rank	
1-3	3.2±0.23	12	3.0±0.09	10	
6-9	1.0±0.03	1	4.2±0.17	2	***
6-15	1.2±0.09	3	4.1±0.11	3	***
7-5	1.1±0.03	2	4.6±0.12	1	***
5-3	1.4±0.17	4	3.6±0.14	6	
6-2	3.1±0.48	11	2.6±0.24	11	
2-4	1.9±0.29	6	3.4±0.16	8	
3-26	2.8±0.26	9	3.5±0.23	7	
3-6	3.0±0.71	10	2.4±0.13	12	
5-36	2.0±0.41	7	3.6±0.43	5	
5-20	1.5±0.29	5	3.4±0.13	9	
6-34	2.8±0.85	8	4.0±0.20	4	
Mean	1.8±0.08		3.8±0.06		
CV	48		19		

***Denotes a top three finish in both categories of psyllid damage and vigor.

Table D.10. Damage and vigor ratings + standard error and rank of self pollinated SELF KX2 families on 24 August 1993. After the first coppice and before the third rogue.

Family	Damage	Rank	Vigor	Rank	
1-3	2.6±0.22	9	3.2±0.16	7	
6-9	1.0±0.00	1	4.8±0.32	2	***
6-15	1.2±0.15	3	3.8±0.25	5	
7-5	1.1±0.07	2	4.3±0.27	3	***
5-3	1.7±0.19	6	3.6±0.24	6	
6-2	1.5±0.34	5	1.8±0.48	11	
2-4	1.8±0.24	7	2.9±0.35	9	
3-26	1.3±0.17	4	2.9±0.36	8	
3-6	1.0±0.00	1	1.0±0.00	12	
5-36	1.3±0.25	4	2.8±1.00	10	
5-20	1.9±0.52	8	4.3±0.48	4	
6-34	1.0±0.00	1	5.2±0.16	1	***
Mean	1.5±0.07		3.6±0.12		
CV	42		29		

***Denotes a top three finish in both categories of psyllid damage and vigor.

Table D.11. Damage and vigor ratings + standard error and rank of self pollinated SELF KX2 families on 28 March 1994. After the second coppice and before the fourth rogue.

Family	Damage	Rank	Vigor	Rank	
1-3	1.9±0.13	6	3.4±0.16	10	
6-9	1.0±0.00	1	4.7±0.25	4	
6-15	1.1±0.10	2	4.1±0.21	6	
7-5	1.0±0.00	1	5.4±0.18	1	***
5-3	1.6±0.15	5	3.7±0.25	8	
6-2	1.0±0.00	1	3.5±0.50	9	
2-4	2.2±0.17	7	3.3±0.49	11	
3-26	1.3±0.21	3	4.0±0.50	7	
3-6	Rogued out of trial				
5-36	1.0±0.00	1	4.8±0.75	3	***
5-20	1.3±0.33	4	4.8±0.44	2	
6-34	1.0±0.00	1	4.7±0.17	5	
Mean	1.4±0.06		4.2±0.12		
CV	26		19		

***Denotes a top three finish in both categories of psyllid damage and vigor.

Table D.12. Damage and vigor ratings + standard error and rank of self pollinated SELF KX2 families on 1 May 1994. After the fourth rogue.

Family	Damage	Rank	Vigor	Rank	
1-3	2.8±0.10	10	3.7±0.18	9	
6-9	1.0±0.00	1	4.7±0.25	4	
6-15	1.3±0.14	4	4.5±0.17	6	
7-5	1.0±0.04	2	5.6±0.15	1	*,***
5-3	1.5±0.22	5	4.0±0.42	7	
6-2	3.0± .	11	3.0± .	10	
2-4	2.0±0.35	8	3.9±0.47	8	
3-26	1.6±0.24	6	4.8±0.14	3	
3-6	Rogued out of trial				
5-36	1.3±0.25	3	4.8±0.75	3	***
5-20	2.2±0.17	9	4.8±0.44	2	
6-34	1.7±0.33	7	4.7±0.16	5	
Mean	1.76±0.09		4.41±0.11		
CV	23		14		

***Denotes a top three finish in both categories of psyllid damage and vigor.

Table D.13. Damage and vigor ratings + standard error and rank of KX3 families on 16 July 1992.

Family	Damage	Rank	Vigor	Rank	
91-1	3.8±0.09	12	2.2±0.05	19	
91-2	3.5±0.10	5	2.6±0.06	9	
91-3	3.7±0.10	11	2.6±0.05	11	
91-5	3.9±0.08	16	2.4±0.08	14	
91-6	3.7±0.08	10	2.7±0.08	6	
91-7	3.9±0.10	15	2.6±0.07	12	
91-8	3.7±0.07	8	2.8±0.07	4	
91-9	4.1±0.08	18	2.3±0.09	16	
91-10	3.9±0.12	14	2.3±0.25	17	
91-11	3.6±0.10	6	2.7±0.05	7	
91-12	2.7±0.19	2	3.1±0.13	2	***
91-13	1.5±0.12	1	3.7±0.12	1	*,***
91-14	4.1±0.09	19	2.6±0.07	10	
91-15	3.4±0.13	3	2.7±0.09	5	***
91-16	3.4±0.28	4	2.9±0.23	3	***
91-17	3.7±0.09	9	2.7±0.06	8	
91-18	3.8±0.10	13	2.3±0.06	18	
91-19	3.6±0.07	7	2.4±0.08	13	
91-20	4.0±0.08	17	2.3±0.10	15	
Mean	3.6±0.04		2.6±0.03		
CV	17		23		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top five finish in both categories of psyllid damage and vigor.

Table D.14. Damage and vigor ratings + standard error and rank of KX3 families on 20 August 1992.

Family	Damage	Rank	Vigor	Rank	
91-1	3.3±0.14	5	2.3±0.05	18	
91-2	4.0±0.13	16	2.7±0.07	5	
91-3	3.9±0.12	12	2.7±0.05	7	
91-5	3.7±0.16	10	2.7±0.09	6	
91-6	3.2±0.51	4	2.9±0.09	4	***
91-7	4.1±0.13	17	2.7±0.06	8	
91-8	4.0±0.10	13	3.0±0.06	3	
91-9	4.0±0.10	15	2.4±0.08	16	
91-10	3.5±0.15	6	2.3±0.05	19	
91-11	3.6±0.16	7	2.7±0.06	12	
91-12	1.6±0.13	2	3.5±0.13	2	***
91-13	1.0±0.03	1	4.1±0.12	1	*.***
91-14	4.3±0.12	19	2.7±0.08	11	
91-15	3.7±0.19	9	2.7±0.09	10	
91-16	2.6±0.35	3	2.7±0.16	9	
91-17	4.1±0.10	18	2.5±0.07	13	
91-18	4.0±0.14	14	2.3±0.06	17	
91-19	3.6±0.13	8	2.4±0.05	14	
91-20	3.8±0.14	11	2.4±0.04	15	
Mean	3.4±0.06		2.7±0.03		
CV	32		16		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top five finish in both categories of psyllid damage and vigor.

Table D.15. Damage and vigor ratings + standard error and rank of KX3 families on 18 Oct 1992.

Family	Damage	Rank	Vigor	Rank	
91-1	4.3±0.12	16	2.6±0.06	17	
91-2	4.2±0.14	14	3.5±0.18	11	
91-3	3.0±0.23	6	3.7±0.12	7	
91-5	2.9±0.26	5	3.6±0.16	8	
91-6	2.1±0.19	3	4.2±0.15	3	***
91-7	3.6±0.21	8	3.5±0.12	10	
91-8	3.7±0.10	9	3.9±0.12	4	
91-9	4.8±0.14	19	2.7±0.10	14	
91-10	4.1±0.20	13	2.5±0.09	18	
91-11	3.7±0.17	10	3.4±0.13	12	
91-12	1.3±0.11	2	4.8±0.18	2	***
91-13	1.0±0.00	1	5.5±0.12	1	*,***
91-14	4.3±0.19	15	3.8±0.17	6	
91-15	2.9±0.58	4	3.6±0.24	9	
91-16	3.3±0.53	3	3.8±0.44	5	***
91-17	4.1±0.17	12	3.0±0.13	13	
91-18	4.6±0.15	18	2.3±0.10	19	
91-19	4.1±0.14	11	2.7±0.07	15	
91-20	4.3±0.13	17	2.6±0.07	16	
Mean	3.5±0.06		3.4±0.05		
CV	27		21		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top five finish in both categories of psyllid damage and vigor.

Table D.16. Damage and vigor ratings + standard error and rank of KX3 from 28 August 1993 on regrowth after coppicing on 13 July 1993. After two rogues.

Family	Damage	Rank	Vigor	Rank	
91-1	2.2±0.17	5	2.1±0.27	17	
91-2	2.3±0.18	6	4.5±0.26	1	*
91-3	2.2±0.19	5	3.9±0.20	8	
91-5	3.3±0.20	12	2.4±0.30	14	
91-6	1.9±0.18	3	3.9±0.23	9	
91-7	2.8±0.26	11	3.2±0.22	11	
91-8	2.6±0.17	9	3.3±0.22	10	
91-9	2.4±0.20	7	3.0±0.24	13	
91-10	2.7±0.20	10	2.3±0.25	16	
91-11	1.9±0.21	4	4.3±0.21	3	***
91-12	1.1±0.08	1	4.2±0.21	4	*,***
91-13	2.2±0.12	5	3.2±0.29	12	
91-14	2.4±0.37	7	4.2±0.24	6	
91-15	2.7±0.20	10	4.2±0.37	7	
91-16	1.6±0.24	2	4.3±0.34	2	***
91-17	2.2±0.12	5	3.2±0.29	12	
91-18	2.5±0.12	8	1.6±0.19	19	
91-19	2.2±0.20	5	2.3±0.20	15	
91-20	2.3±0.32	6	1.8±0.22	18	
Mean	2.2±0.05		3.1±0.07		
CV	38		32		

*Denotes the best ranking for either psyllid damage or vigor
***Denotes a top five finish in both categories of psyllid damage and vigor.

Table D.17. Damage and vigor ratings + standard error and rank of KX3 families from 28 March 1994 on regrowth after coppicing on 6 February 1994. After three rogues.

Family	Damage	Rank	Vigor	Rank	
91-1	3.3±0.21	13	2.3±0.31	16	
91-2	3.4±0.17	15	4.5±0.28	4	
91-3	3.1±0.15	9	3.7±0.19	9	
91-5	3.2±0.12	12	3.9±0.37	7	
91-6	2.8±0.14	4	4.1±0.26	6	
91-7	3.1±0.18	10	3.7±0.17	11	
91-8	3.6±0.15	17	3.9±0.23	8	
91-9	3.2±0.15	11	3.7±0.21	12	
91-10	3.0±0.32	8	3.5±0.32	14	
91-11	3.5±0.11	16	4.6±0.17	3	
91-12	1.8±0.17	2	5.3±0.20	2	***
91-13	1.0±0.0	1	5.5±0.15	1	*,***
91-14	2.8±0.14	4	4.3±0.21	5	***
91-15	2.8±0.32	4	4.3±0.32	5	***
91-16	2.1±0.33	3	4.5±0.45	4	***
91-17	3.3±0.21	14	3.6±0.36	13	
91-18	3.7±0.20	18	3.7±0.54	10	
91-19	2.9±0.19	7	3.5±0.32	15	
91-20	4.1±0.10	19	2.5±0.58	17	
Mean	2.8±0.07		4.1±0.08		
CV	18		20		

*Denotes the best ranking for either psyllid damage or vigor

***Denotes a top five finish in both categories of psyllid damage and vigor.

Table D.18. Damage and vigor ratings + standard error and rank of KX3 families from 1 May 1994 on regrowth after coppicing on 6 February 1994. After four rogues.

Family	Damage	Rank	Vigor	Rank	
91-1	3.3±0.13	10	2.8±0.20	16	
91-2	3.3±0.16	12	4.8±0.17	3	
91-3	3.0±0.16	8	3.9±0.20	13	
91-5	3.2±0.13	10	4.3±0.14	9	
91-6	2.7±0.18	5	4.4±0.31	8	
91-7	3.1±0.24	11	3.9±0.14	14	
91-8	3.5±0.15	13	4.2±0.18	10	
91-9	3.1±0.24	9	4.1±0.20	12	
91-10	3.0±0.33	7	3.6±0.44	15	
91-11	3.5±0.11	13	4.6±0.17	4	
91-12	1.8±0.18	2	5.4±0.18	2	***
91-13	1.0±0.0	1	5.7±0.10	1	*,***
91-14	2.7±0.17	5	4.4±0.19	6	
91-15	2.7±0.32	6	4.3±0.33	9	
91-16	2.1±0.33	3	4.4±0.45	5	***
91-17	3.0±0.16	8	4.2±0.24	11	
91-18	3.5±0.29	13	4.2±0.29	5	
91-19	2.5±0.26	4	4.4±0.26	7	
91-20	4.3± .	14	2.2± .	17	
Mean	2.91±0.08		4.19±0.07		
CV	18		13		

*Denotes the best ranking for either psyllid damage or vigor
***Denotes a top five finish in both categories of psyllid damage and vigor.

APPENDIX E

Mean height and diameter of 24 *Leucaena* genotypes grown for 3 years in SET 91-3 at Waimanalo.

Table E.1. Total height (m) after 1, 2 and 3 years with standard error for the total of 24 *Leucaena* genotypes in SET 91-3 Waimanalo, Hawaii.

	YEAR			
Genotype	1	2	3	Total
K636	4.27	2.69	1.27	8.23±0.40
KX3	3.15	2.94	1.86	7.95±0.43
K784	3.69	2.45	1.72	7.86±0.19
K584	3.63	2.46	1.69	7.79±0.30
K902	4.11	1.90	1.53	7.54±0.34
K397	3.82	2.47	1.20	7.49±0.35
K565	3.88	2.03	1.42	7.33±0.29
K608	3.52	2.18	1.54	7.24±0.26
KX2	2.98	2.12	2.04	7.14±0.35
K925	4.10	1.58	1.26	6.94±0.25
KX1	3.43	1.97	1.45	6.85±0.31
K638	2.82	2.40	1.40	6.62±0.31
K419	3.24	2.04	1.27	6.55±0.36
K952	3.36	1.72	1.20	6.28±0.30
K633	1.95	2.23	1.88	6.06±0.29
K29	2.12	2.33	1.56	6.01±0.32
K418	2.76	2.13	0.96	5.85±0.28
K156	1.72	2.21	1.89	6.38±0.45
K67	2.05	2.16	1.48	5.85±0.36
K678	2.52	1.79	1.23	5.54±0.43
K665	1.40	2.85	1.14	5.40±0.55
K217	2.66	1.93	0.66	5.40±0.24
K417	1.91	1.97	1.15	5.03±0.37
K21	0.90	1.40	1.05	3.35±0.60
Average interval	2.91	2.20	1.52	6.61±0.7

Table E.2. Total diameter (mm) after 1, 2 and 3 years with standard error for the total of 24 *Leucaena* genotypes in SET 91-3 at Waimanalo, Hawaii.

	YEAR			
Genotype	1	2	3	Total
K636	30.0	24.0	11.8	65.8±0.40
KX3	26.1	19.4	17.2	62.7±0.43
K902	31.0	12.3	12.7	56.0±0.34
KX2	23.8	18.1	13.6	55.5±0.35
K784	23.7	18.4	10.4	52.5±0.19
K584	24.5	16.1	11.4	52.0±0.30
K608	25.5	16.5	9.4	51.4±0.26
K397	23.4	17.9	9.3	50.6±0.35
K565	25.6	15.0	9.5	50.1±0.29
KX1	23.9	12.8	10.3	47.0±0.31
K638	17.9	14.3	10.0	42.2±0.31
K952	22.1	10.8	5.2	38.1±0.30
K925	22.3	12.0	3.8	38.1±0.25
K419	19.6	9.8	7.9	37.3±0.36
K156	11.6	11.5	11.5	34.6±0.45
K29	11.0	15.2	6.3	32.5±0.32
K678	15.6	8.2	7.2	31.0±0.43
K633	11.3	11.1	8.2	30.6±0.29
K418	14.2	11.0	4.2	29.4±0.28
K67	11.8	13.1	4.4	29.3±0.36
K665	14.2	8.2	3.5	28.9±0.55
K217	15.4	8.2	3.5	27.1±0.24
K417	10.5	8.2	5.3	24.0±0.37
K21	5.5	6.5	7.2	19.2±0.60
Average interval	20.4	12.6	8.9	41.9±0.87

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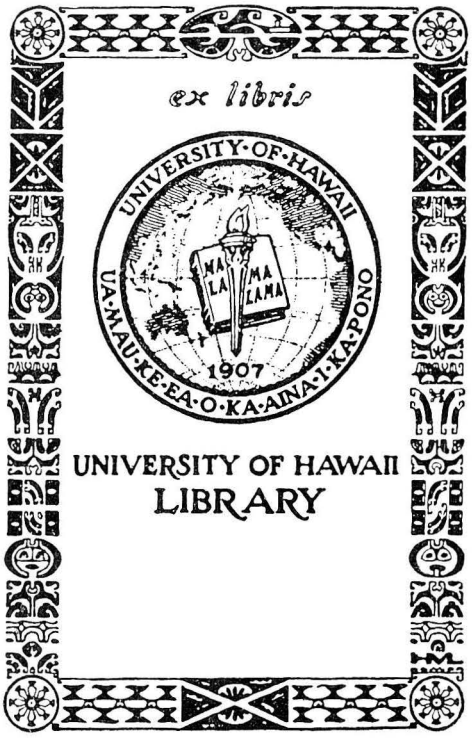
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